

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|--|-----------|---|
| (51) International Patent Classification ⁶: A61K 39/395, C07K 16/18, 16/28 | A1 | (11) International Publication Number: WO 98/19706 (43) International Publication Date: 14 May 1998 (14.05.98) |
| (21) International Application Number: PCT/US97/19906 (22) International Filing Date: 29 October 1997 (29.10.97) (30) Priority Data: 08/746,361 8 November 1996 (08.11.96) US (71) Applicant: IDEC PHARMACEUTICALS CORPORATION [US/US]; 11011 Torreyana Road, San Diego, CA 92121 (US). (72) Inventors: ANDERSON, Darrell, R.; 1851 Navajo Place, Escondido, CA 92029 (US). HANNA, Nabil; 3255 Fortuna Ranch Road, Olivenhain, CA 92024 (US). BRAMS, Peter; 4260 3rd Avenue #101, San Diego, CA 92103 (US). (74) Agents: GESS, E., Joseph et al.; Burns, Doane, Swecker & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US). | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> |
| (54) Title: IDENTIFICATION OF UNIQUE BINDING INTERACTIONS BETWEEN CERTAIN ANTIBODIES AND THE HUMAN B7.1 AND B7.2 CO-STIMULATORY ANTIGENS (57) Abstract The present invention relates to the identification of antibodies which are specific to human B7.1 antigen (CD80) and which are capable of inhibiting the binding of B7.1 to a CD28 receptor and which are not capable of inhibiting the binding of B7.1 to a CTLA-4 receptor. Two of these antibodies, 16C10 and 7C10, significantly inhibit the production of IL-2, in spite of the existence of a second activating ligand B7.2 (CD86). Blocking of the primary activation signal between CD28 and B7.1 (CD80) with these antibodies while allowing the unimpaired or coincident interaction of CTLA-4 and B7.1 and/or B7.2 represents a combined antagonistic effect on positive co-stimulation with an agonistic effect on negative signalling. These antibodies may be used as specific immunosuppressants, e.g., for the treatment of autoimmune diseases and to prevent organ transplant rejection. | | |

AN

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | ML | Mali | TR | Turkey |
| BG | Bulgaria | HU | Hungary | MN | Mongolia | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MR | Mauritania | UA | Ukraine |
| BR | Brazil | IL | Israel | MW | Malawi | UG | Uganda |
| BY | Belarus | IS | Iceland | MX | Mexico | US | United States of America |
| CA | Canada | IT | Italy | NE | Niger | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NL | Netherlands | VN | Viet Nam |
| CG | Congo | KE | Kenya | NO | Norway | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NZ | New Zealand | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | PL | Poland | | |
| CM | Cameroon | KR | Republic of Korea | PT | Portugal | | |
| CN | China | KZ | Kazakstan | RO | Romania | | |
| CU | Cuba | LC | Saint Lucia | RU | Russian Federation | | |
| CZ | Czech Republic | LI | Liechtenstein | SD | Sudan | | |
| DE | Germany | LK | Sri Lanka | SE | Sweden | | |
| DK | Denmark | LR | Liberia | SG | Singapore | | |
| EE | Estonia | | | | | | |

-1-

**IDENTIFICATION OF UNIQUE BINDING INTERACTIONS BETWEEN
CERTAIN ANTIBODIES AND THE HUMAN B7.1 AND B7.2
CO-STIMULATORY ANTIGENS**

FIELD OF THE INVENTION

5 The present invention relates to the identification
and use of monoclonal antibodies which are specific to
B7.1 antigens (CD80). More specifically, the present
invention relates to the identification and use of
monoclonal antibodies or primatized forms thereof which
10 are capable of inhibiting the binding of human B7.1
antigen to a CD28 receptor and which are not capable of
inhibiting the binding of B7.1 to a CTLA-4 receptor.
Thus, the invention relates to the identification and
use of monoclonal antibodies and primatized forms
15 thereof which recognize specific sites on the B7.1
antigen which are exclusive of CTLA-4 receptor binding.

 The invention further relates to monoclonal
antibodies or primatized forms thereof which recognize
specific sites on the human B7.1 antigen and are capable
20 of inhibiting IL-2 production.

 Also, the present invention relates to
pharmaceutical compositions containing monoclonal or
primatized antibodies specific to human B7.1 and their
use as immunosuppressants by modulating the B7:CD28
25 pathway, e.g., for the treatment of autoimmune
disorders, and the prevention of organ rejection.

BACKGROUND OF THE INVENTION

 The clinical interface between immunology,
hematology, and oncology has long been appreciated.
30 Many conditions treated by the hematologist or
oncologist have either an autoimmune or immunodeficient

-2-

component to their pathophysiology that has led to the widespread adoption of immunosuppressive medications by hematologists, whereas oncologists have sought immunologic adjuvants that might enhance endogenous
5 immunity to tumors. To date, these interventions have generally consisted of nonspecific modes of immunosuppression and immune stimulation. In addition to the limited efficacy of these interventions, toxicities secondary to their nonspecificity have also
10 limited their overall success. Therefore, alternative strategies have been sought.

Elucidation of the functional role of a rapidly increasing number of cell surface molecules has contributed greatly to the integration of immunology
15 with clinical hematology and oncology. Nearly 200 cell surface antigens have been identified on cells of the immune and hematopoietic systems (Schlossman SF, Boumsell L, Gilks JM, Harlan T, Kishimoto, C Morimoto C, Ritz J., Shaw S, Silverstein RL, Springer TA, Tedder TF,
20 Todd RF:CD antigens (1993), Blood 83:879, 1994). These antigens represent both lineage-restricted and more widely distributed molecules involved in a variety of processes, including cellular recognition, adhesion, induction and maintenance of proliferation, cytokine
25 secretion, effector function, and even cell death. Recognition of the functional attributes of these molecules has fostered novel attempts to manipulate the immune response. Although molecules involved in cellular adhesion and antigen-specific recognition have
30 previously been evaluated as targets of therapeutic immunologic intervention, recent attention has focused on a subgroup of cell surface molecules termed co-stimulatory molecules (Bretscher P: "The two-signal model of lymphocyte activation twenty-one years later."

-3-

- Immunol. Today 13:73, (1992); Jenkins MK, Johnson JG: "Molecules involved in T-cell co-stimulation." Curr Opin Immunol 5:351, (1993); Geppert T, Davis L. Gur H. Wacholtz M. Lipsky P: "Accessory cell signals involved in T-cell activation." Immunol Rev 117:5, (1990); Weaver CT, Unanue ER: "The co-stimulatory function of antigen-presenting cells." Immunol Today 11:49, (1990); Stennam RM, Young JW: "Signals arising from antigen-presenting cells." Curr Opin Immunol 3:361, (1991)).
- 10 Co-stimulatory molecules do not initiate but rather enable the generation and amplification of antigen-specific T-cell responses and effector function (Bretscher P: "The two-signal model of lymphocyte activation twenty-one years later." Immunol. Today 13:73, (1992); Jenkins MK, Johnson JG: "Molecules involved in T-cell co-stimulation." Curr Opin Immunol 5:351, (1993); Geppert T, Davis L. Gur H. Wacholtz M. Lipsky P: "Accessory cell signals involved in T-cell activation." Immunol Rev 117:5, (1990); Weaver CT, Unanue ER: "The co-stimulatory function of antigen-presenting cells." Immunol Today 11:49, (1990); Stennam RM, Young JW: "Signals arising from antigen-presenting cells." Curr Opin Immunol 3:361, (1991); June CH, Bluestone JA, Linsley PS, Thompson CD: "Role of the CD28 receptor in T-cell activation." Immunol Today 15:321, (1994)).
- 25

Recently, one specific co-stimulatory pathway termed B7:CD28 has been studied by different research groups because of its significant role in B and T cell activation (June CH, Bluestone JA, Linsley PS, Thompson CD: "Role of the CD28 receptor in T-cell activation." Immunol Today 15:321, (1994); June CH, Ledbetter JA: "The role of the CD28 receptor during T-cell responses to antigen." Annu Rev Immunol 11:191, (1993); Schwartz

30

-4-

RH: "Co-stimulation of T lymphocytes: The role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy." Cell 71:1065-1068, (1992); Jenkins MK, Taylor PS, Norton SD, Urdahl KB: "CD28 delivers a
5 ~~costimulatory signal involved in antigen-specific~~ IL-2
production by human T cells." Journal of Immunology
147:2461-2466 (1991)). Since this ligand:receptor
pathway was discovered four years ago, a large body of
evidence has accumulated suggesting that B7:CD28
10 interactions represent one of the critical junctures in
determining immune reactivity versus anergy (June CH,
Bluestone JA, Linsley PS, Thompson CD: "Role of the CD28
receptor in T-cell activation." Immunol Today 15:321,
(1994); June CH, Ledbetter JA: "The role of the CD28
15 receptor during T-cell responses to antigen." Annu Rev
Immunol 11:191, (1993); Schwartz RH: "Co-stimulation of
T lymphocytes: The role of CD28, CTLA-4, and B7/BB1 in
interleukin-2 production and immunotherapy." Cell
71:1065-1068, (1992); Cohen J: "Mounting a targeted
20 strike on unwanted immune responses" (news; comment).
Science 257:751, (1992); Cohen J: "New protein steals
the show as 'co-stimulator' of T cells" (news; comment).
Science 262:844, (1993)).

In particular, the role of the human B7 antigens,
25 i.e., human B7.1 (CD80) and B7.2 (CD86), has been
reported to play a co-stimulatory role in T-cell
activation. See, e.g., Gimmi CD, Freeman, GJ, Gribben
JG, Sugita K, Freedman AS, Morimoto C, Nadler LM: "B-
cell surface antigen B7 provides a costimulatory signal
30 that induces T cells to proliferate and secrete
interleukin 2." Proc. Natl. Acad. Sci. (USA) 88:6575-
6579 (1991).

1. B7.1 and B7.2 Co-stimulatory Role in T Cell Activation

-5-

The elaboration of a successful immune response depends on a series of specific interactions between a T cell and an antigen presenting cell. Although the essential first step in this process depends upon the

5 binding of antigen to the T cell receptor, in the context of the MHC class II molecule (Lane, P.J.L., F.M. McConnell, G.L. Schieven, E.A. Clark, and J.A. Ledbetter, (1990), "The Role of Class II Molecules in Human B Cell Activation." The Journal of Immunology, 144:3684-3692), this interaction alone is not sufficient

10 to induce all the events necessary for a sustained response to a given antigen (Schwartz, R.H. (1990), "A Cell Culture Model for T Lymphocyte Clonal Anergy." Science, 248:1349; Jenkins, M.K. (1992), "The Role of

15 Cell Division in the Induction of Clonal Anergy." Immunology Today, 13:69; Azuma, M., M. Cayabyab, D. Buck, J.H. Phillips, and L.L. Lanier, (1992), "Involvement of CD28 in MHC-unrestricted Cytotoxicity Mediated by a Human Natural Killer Leukemia Cell Line."

20 The Journal of Immunology, 149:1115-1123; Azuma, M., M. Cayabyab, D. Buck, J.H. Phillips, and L.L. Lanier, (1992), "CD28 Interaction with B7 Costimulates Primary Allogeneic Proliferative Responses and Cytotoxicity Mediated by Small Resting T Lymphocytes." J. Exp. Med., 175:353-360); S.D. Norton, L. Zuckerman, K.B. Urdahl, R. Shefner, J. Miller, and M.K. Jenkins, (1992), "The CD28

25 Ligand, B7, Enhances IL-2 Production by Providing a Costimulatory Signal to T Cells." The Journal of Immunology, 149:1556-1561; R. H. Schwartz, (1992),

30 "Costimulation of T Lymphocytes: The Role of CD28, CTLA-4, and B7/BB1 in Interleukin-2 Production and Immunotherapy." Cell 71:1065-1068).

The involvement of certain other co-stimulatory molecules is necessary (Norton, S.D., L. Zuckerman, K.B.

-6-

- Urdahl, R. Shefner, J. Miller, and M.K. Jenkins, (1992), "The CD28 Ligand, B7, Enhances IL-2 Production by Providing A Costimulatory Signal to T Cells." The Journal of Immunology, 149:1556-1561). "The homodimers
- 5 CD28 and CTLA-4 expressed on T-cells" (June, C.H., J.A. Ledbetter, P.S. Linsley, and C.B. Thompson, (1990), "Role of the CD28 Receptor in T-Cell Activation." Immunology Today, 11:211-216; Linsley, P.S., W. Brady, M. Urnes, L.S. Grosmaire, N.K. Damle, and J.A.
- 10 Ledbetter, (1991), "CTLA-4 is a Second Receptor for the B Cell Activation Antigen B7." J. Exp. Med., 174:561), together with B7.1 (CD80) and B7.2 (CD86) expressed on antigen presenting cells, are major pairs of co-
- 15 stimulatory molecules necessary for a sustained immune response (Azuma, M., H. Yssel, J.H. Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes." J. Exp. Med., 177:845-850; Freeman, G.J., A.S. Freedman, J.M. Segil, G. Lee, J.F. Whitman, and LM. Nadler, (1989), "B7, A
- 20 New Member of the Ig Superfamily with Unique Expression on Activated and Neoplastic B Cells." The Journal of Immunology, 143:2714-2722; Hathcock, K.S., G. Laslo, H.B. Dickler, J. Bradshaw, P. Linsley, and R.J. Hodes, (1993), "Identification of an Alternative CTLA-4 Ligand
- 25 Costimulatory for T Cell Activation." Science, 262:905-911; Hart, D.N.J., G.C. Starling, V.L. Calder, and N.S. Fernando, (1993). "B7/BB-1 is a Leucocyte
- 30 Differentiation Antigen on Human Dendritic Cells Induced by Activation." Immunology, 79:616-620). It can be shown in vitro that the absence of these co-stimulatory signals leads to an aborted T cell activation pathway and the development of unresponsiveness to the specific antigen, or anergy. (See, e.g., Harding, F.A., J.G. McArthur, J.A. Gross, D.M. Raulet, and J.P. Allison,

-7-

- (1992), "CD28 Mediated Signalling Co-simulates Murine T Cells and Prevents Induction of Anergy in T Cell Clones." Nature, 356:607-609; Gimmi, C.D., G.J. Freeman, J.G. Gribben, G. Gray, and L.M. Nadler, (1993);
- 5 "Human T-Cell Clonal Anergy is Induced by Antigen Presentation in the Absence of B7 Costimulation." Proc. Natl. Acad. Sci., 90:6586-6590; Tan, P., C. Anasefti, J.A. Hansen, J. Melrose, M. Brunvand, J. Bradshaw, J.A. Ledbetter, and P.S. Linsley, (1993), "Induction of
- 10 Alloantigen-specific Hyporesponsiveness in Human T Lymphocytes by Blocking Interaction of CD28 with Its Natural Ligand B7/BB1." J. Exp. Med., 177:165-173). Achievement of *in vivo* tolerance constitutes a mechanism for immunosuppression and a viable therapy for organ
- 15 transplant rejection and for the treatment of autoimmune diseases. This has been achieved in experimental models following the administration of CTLA-4Ig (Lenschow, D.J., Y. Zeng, R.J. Thistlethwaite, A. Montag, W. Brady, M.G. Gibson, P.S. Linsley, and J.A. Bluestone, (1992),
- 20 "Long-Term Survival of Xenogeneic Pancreatic Islet Grafts Induced by CTLA-4Ig." Science, 257:789-795).
- The molecules B7.1 and B7.2 can bind to either CD28 or CTLA-4, although B7.1 binds to CD28 with a Kd of 200
- 25 (Linsley, P.S., E.A. Clark, and J.A. Ledbetter, (1990), "T-Cell Antigen CD28 Mediates Adhesion with B Cells by Interacting with Activation Antigen B7/BB-1." Proc. Natl. Acad. Sci., 87:5031-5035; Linsley et al, (1993), "The Role of the CD28 receptor during T cell responses
- 30 to antigen," Annu. Rev. Immunol., 11:191-192; Linesley et al, (1993), "CD28 Engagement by B7/BB-1 Induces Transient Down-Regulation of CD28 Synthesis and Prolonged Unresponsiveness to CD28 Signaling," The Journal of Immunology, 150:3151-3169). B7.1 is

-8-

expressed on activated B cells and interferon induced monocytes, but not resting B cells (Freeman, G.J., G.S. Gray, C.D. Gimmi, D.B. Lomarrd, L-J. Zhou, M. White, J.D. Fingerioth, J.G. Gribben, and L.M. Nadler, (1991).

- 5 ~~"Structure, Expression and T-Cell Costimulatory Activity~~
of the Murine Homologue of the Human B Lymphocyte
Activation Antigen B7," J. Exp. Med., 174:625-631).
B7.2, on the other hand, is constitutively expressed at
very low levels on resting monocytes, dendritic cells
10 and B cells, and its expression is enhanced on activated
T cells, NK cells and B lymphocytes (Azuma, M. D. Ito,
H. Yagita, K. Okumura, J.H. Phillips, L.L. Lanier, and
C. Somoza, 1993, "B70 Antigen is a Second Ligand for
CTLA-4 and CD28," Nature, 366:76-79). Although B7.1
15 and B7.2 can be expressed on the same cell type, their
expression on B cells occurs with different kinetics
(Lenschow, D.J., G.H. Su, L.A. Zuckerman, N. Nabavi,
C.L. Jellis, G.S. Gray, J. Miller, and J.A. Bluestone,
(1993), "Expression and Functional Significance of an
20 Additional Ligand for CTLA-4," Proc. Natl. Acad. Sci.,
USA, 90:11054-11058; Boussiotis, V.A., G.J. Freeman,
J.G. Gribben, J. Daley, G. Gray, and L.M. Nadler,
(1993), "Activated Human B Lymphocytes Express Three
CTLA-4 Counter-receptors that Co-stimulate T-Cell
25 Activation." Proc. Natl. Acad. Sci., USA, 90:11059-
11063). Further analysis at the RNA level has
demonstrated that B7.2 mRNA is constitutively expressed,
whereas B7.1 mRNA is detected 4 hours after activation
and initial low levels of B7.1 protein are not
30 detectable until 24 hours after stimulation (Boussiotis,
V.A., G.J. Freeman, J.G. Gribben, J. Daley, G. Gray, and
L.M. Nadler, (1993), "Activated Human B Lymphocytes
Express Three CTLA-4 Counter-receptors that Co-stimulate
T-Cell Activation," Proc. Natl. Acad. Sci., USA,

-9-

90:11059-11063). CTLA-4/CD28 counter receptors, therefore, may be expressed at various times after B Cell activation.

More recently, it has been suggested that the
5 ~~second T cell associated co-receptor CTLA-4~~ apparently
functions as a negative modulator to override and
prevent a runaway immune system (Krummel M, Allison J:
"CD28 and CTLA-4 have opposing effects on the response
of T cells to stimulation." J. Exp. Med. 182:459-466
10 (1995)). The CTLA-4 receptor plays a critical role in
down regulating the immune response, as evidenced in
CTLA-4 knockout mice. Knockout mice born without the
ability to express the CTLA-4 gene die within 3-4 weeks
of severe lymphoproliferative disorder (Tivol EA,
15 Borriello G, Schweitzer AN, Lynch WP, Bluestone JA,
Sharpe AH: "Loss of CTLA-4 leads to massive
lymphoproliferation and fatal multiorgan tissue
destruction, revealing a critical negative regulatory
role of CTLA-4." Immunity 3:541-547 (1995)). CTLA-4 is
20 thought to function through signaling mechanisms linked
to induction of apoptosis (Gribben JG, Freeman GJ,
Boussiotis VA, Rennert P, Jellis CL, Greenfield E,
Barber M, Restivo Jr. VA, Ke X, Gray GS, Nadler LM:
"CTLA-4 mediates antigen specific apoptosis of human T
25 cells." Proc. Natl. Acad. Sci. (USA) 92:811-815 (1995)),
triggered through as yet undefined ligand binding to
specific sites on the receptor. It has been shown *in*
vitro that the blocking of the B7.1/B7.2 dependent co-
stimulatory signals in various ways leads to an aborted
30 T cell activating pathway and the development of
unresponsiveness to the specific antigen (Lederman S,
Chess L, Yellin MJ: "Murine monoclonal antibody (5c8)
recognizes a human glycoprotein on the surface of T-
lymphocytes, compositions containing same." U.S. Patent

-10-

- No. 5,474,771 (December 12, 1995); Linsley PS, Ledbetter JA, Damle NK, Brady W: "Chimeric CTLA4 receptor and methods for its use." U.S. Patent No. 5,434,131 (July 18, 1995); Harding, 1992; Gimmi CD, Freeman GJ, Bribben
- 5 JG, Gray G, Nadler LM: "Human-T-cell-clonal-anergy is induced by antigen presentation in the absence of B7 costimulation." Proc. Natl. Acad. Sci. (USA) 90:6586-6590 (1993); Tan P, Anasetti C, Hansen JA, Melrose J, Brunvand M, Bradshaw J, Ledbetter JA, Linsley PS:
- 10 "Induction of alloantigen-specific hyporesponsiveness in human T lymphocytes by blocking interaction of CD28 with its natural ligand B7/BB1." J. Exp. Med. 177:165-173 (1993)). Achievement of *in vivo* tolerance, anergy, or depleting of antigen-specific T cells would constitute a
- 15 mechanism for immunosuppression and a viable therapy for organ transplant rejection or plausible treatment for autoimmune diseases.

- The differential temporal expression of B7.1 and B7.2 suggests that the interaction of these two
- 20 molecules with CTLA-4 and/or CD28 deliver distinct but related signals to the T cell (LaSalle, J.M., P.J. Tolentino, G.J. Freeman, L.M. Nadler, and D.A. Hafler, (1992), "CD28 and T Cell Antigen Receptor Signal Transduction Coordinately Regulate Interleukin 2 Gene
- 25 Expression In Response to Superantigen Stimulation," J. Exp. Med., 176:177-186; Vandenberghe, P., G.J. Freeman, L.M. Nadler, M.C. Fletcher, M. Kamoun, L.A. Turka, J.A. Ledbetter, C.B. Thompson, and C.H. June, (1992), "Antibody and B7/BB1-mediated Ligation of the CD28
- 30 Receptor Induces Tyrosine Phosphorylation in Human T Cells," The Journal of Experimental Medicine, 175:951-960). The exact signaling functions of CTLA-4 and CD28 on the T cell are currently unknown (Janeway, C.A., Jr. and K. Bottomly, (1994), "Signals and Signs

-11-

for Lymphocyte Responses," Cell, 76:275285). However, it is possible that one set of receptors could provide the initial stimulus for T cell activation and the second, a sustained signal to allow further elaboration of the pathway and clonal expansion to take place (Linsley, P.S., J.L. Greene, P. Tan, J. Bradshaw, J.A. Ledbetter, C. Anasetti, and N.K. Damle, (1992), "Coexpression and Functional Cooperation of CTLA-4 and CD28 on Activated T Lymphocytes," J. Exp. Med., 176:1595-1604). The current data supports the two-signal hypothesis proposed by Jenkins and Schwartz (Schwartz, R.H., (1990), "A Cell Culture Model for T Lymphocyte Clonal Anergy," Science, 248:1349; Jenkins, M.K., (1992), "The Role of Cell Division in the Induction of Clonal Anergy," Immunology Today, 13:69) that both a TCR and co-stimulatory signal are necessary for T cell expansion, lymphokine secretion and the full development of effector function (Greenan, V. and G. Kroemer, (1993), "Multiple Ways to Cellular Immune Tolerance," Immunology Today, 14:573). The failure to deliver the second signal results in the inability of T cells to secrete IL-2 and renders the cell unresponsive to antigen.

Structurally, both B7.1 and B7.2 contain extracellular immunoglobulin superfamily V and C-like domains, a hydrophobic transmembrane region and a cytoplasmic tail (Freeman, G.J., J.G. Gribben, V.A. Boussiotis, J.W. Ng, V. Restivo, Jr., L.A. Lombard, G.S. Gray, and L.M. Nadler, (1993), "Cloning of B7.2: A CTLA-4 Counter-receptor that Co-stimulates Human T Cell Proliferation," Science, 262:909). Both B7.1 and B7.2 are heavily glycosylated. B7.1 is a 44-54kD glycoprotein comprised of a 223 amino acid extracellular domain, a 23 amino acid transmembrane domain, and a 61

-12-

amino acid cytoplasmic tail. B7.1 contains 3 potential protein kinase phosphorylation sites. (Azuma, M., H. Yssel, J.H. Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes," J. Exp. Med., 177:845-850). B7.2 is a 306 amino acid membrane glycoprotein. It consists of a 220 amino acid extracellular region, a 23 amino acid hydrophobic transmembrane domain and a 60 amino acid cytoplasmic tail (Freeman, G.J., A.S. Freedman, J.M. Segil, G. Lee, J.F. Whitman, and LM. Nadler, (1989), "B7, A New Member of the Ig Superfamily with Unique Expression on Activated and Neoplastic B Cells," The Journal of Immunology, 143:2714-2722). Although both B7.1 and B7.2 genes are localized in the same chromosomal region (Freeman, G.J., D.B. Lombard, C.D. Gimmi, S.A. Brod, L Lee, J.C. Laning, D.A. Hafler, M.E. Dorf, G.S. Gray, H. Reiser, C.H. June, C.B. Thompson, and L.M. Nadler, (1992), "CTLA-4 and CD28 mRNA are Coexpressed in Most T Cells After Activation," The Journal of Immunology, 149:3795-3801; Schwartz, R.H., (1992), "Costimulation of T Lymphocytes: The Role of CD28, CTLA-4, and B7/BB1" in Selvakumar, A., B.K. Mohanraj, R.L. Eddy, T.B. Shows, P.C. White, C. Perrin, and B. Dupont, (1992), "Genomic Organization and Chromosomal Location of the Human Gene Encoding the B-Lymphocyte Activation Antigen B7," Immunogenetics, 36:175-181), these antigens do not share a high level of homology. The overall homology between B7.1 and B7.2 is 26% and between murine B7.1 and human B7.1 is 27% (Azuma, M., H. Yssel, J.H. Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes," J. Exp. Med., 177:845-850; Freeman, G.J., A.S. Freedman, J.M. Segil, G. Lee, J.F. Whitman, and LM. Nadler, (1989), "B7, A New Member of

-13-

the Ig Superfamily with Unique Expression on Activated and Neoplastic B Cells," The Journal of Immunology, 143:2714-2722). Although alignment of human B7.1 human B7.2 and murine B7.1 sequences shows few stretches of
5 lengthy homology, ~~it is known that all three molecules~~
bind to human CTLA-4 and CD28. Thus, there is most likely a common, or closely homologous region shared by the three molecules that may be either contiguous or conformational. This region may constitute the binding
10 site of the B7.1 and B7.2 molecules to their counter-receptors. Antibodies raised against these epitopes could potentially inhibit the interaction of B7 with its counter-receptor on the T cell. Furthermore, antibodies that cross-reacted with this region on both B7.1 and
15 B7.2 molecules would potentially have practical advantages over antibodies directed against B7.1 or B7.2 separately.

2. Blockade of the B7/CD28 Interaction

Blocking of the B7/CD28 interaction offers the
20 possibility of inducing specific immunosuppression, with potential for generating long lasting antigen-specific therapeutic effects. Antibodies or agents that temporarily prevent this interaction may be useful, specific and safe clinical immunosuppressive agents,
25 with potential for generating long term antigen-specific therapeutic effects.

Antibodies to either B7.1 or B7.2 have been shown to block T cell activation, as measured by the inhibition of IL-2 production in vitro (DeBoer, M., P.
30 Parren, J. Dove, F. Ossendorp, G. van der Horst, and J. Reeder, (1992), "Functional Characterization of a Novel Anti-B7 Monoclonal Antibody," Eur. Journal of Immunology, 22:3071-3075; Azuma, M., H. Yssel, J.H.

-14-

Phillips, H. Spits, and L.L. Lanier, (1993), "Functional Expression of B7/BB1 on Activated T Lymphocytes," J. Exp. Med., 177:845-850). However, different antibodies have been shown to vary in their immunosuppressive potency, which may reflect either their affinity or epitope specificity. A possible explanation for this may reside in the ability of some antibodies to block only the binding of B7 to CD28, while promoting apoptosis or some other form of negative signaling through the CTLA-4 receptor in activated T cells. Some antibodies to B7.1 or B7.2 may, in fact, hinder the activity of CTLA-4 by cross-reacting with the CTLA-4 binding domain. CTLA-4Ig fusion protein and anti-CD28 Fabs were shown to have similar effects on the down regulation of IL-2 production.

In vivo administration of a soluble CTLA-4Ig fusion protein has been shown to suppress T cell dependent antibody responses in mice (Linsley, P.S., J.L. Greene, P. Tan, J. Bradshaw, J.A. Ledbetter, C. Anasetti, and N.K. Damle, (1992), "Coexpression and Functional Cooperation of CTLA-4 and CD28 on Activated T Lymphocytes," J. Exp. Med., 176:1595-1604; Lin, H., S.F. Builing, P.S. Linsley, R.O. Wei, C.D. Thompson, and L.A. Turka, (1993), "Long-term Acceptance of Major Histocompatibility Complex Mismatched Cardiac Allografts Induced by CTLA-4-Ig Plus Donor Specific Transfusion," J. Exp. Med., 178:1801) and, furthermore, larger doses were also able to suppress responses to a second immunization, demonstrating the feasibility of this approach for the treatment of antibody mediated autoimmune disease. In addition, CTLA-4Ig was able to prevent pancreatic islet cell rejection in mice by directly inhibiting the interaction of T cells and B7.1/B7.2 antigen presenting cells (Lenschow, D.J., G.H.

-15-

Su, L.A. Zuckerman, N. Nabavi, C.L. Jellis, G.S. Gray, J. Miller, and J.A. Bluestone, (1993), "Expression and Functional Significance of an Additional Ligand for CTLA-4," Proc. Natl. Acad. Sci., USA, 90:11054-11058).

5 — In this case, long term donor specific tolerance was achieved.

3. Recombinant Phage Display Technology for Antibody Selection

To date, no monoclonal antibodies which crossreact
10 with both B7.1 and B7.2 have been reported.
Furthermore, no monoclonal antibodies which are specific to B7.1 or B7.2 and which also recognize specific sites on the antigens which are restricted to co-activation receptor CD28 binding have been reported. Or
15 alternatively, no monoclonal antibodies which are specific to B7.1 or B7.2 and which recognize specific sites on the antigens which are exclusive of CTLA-4 receptor binding have been reported. As discussed supra, such antibodies would potentially be highly
20 desirable as immunosuppressants.

Phage display technology is beginning to replace traditional methods for isolating antibodies generated during the immune response, because a much greater percentage of the immune repertoire can be assessed than
25 is possible using traditional methods. This is in part due to PEG fusion inefficiency, chromosomal instability, and the large amount of tissue culture and screening associated with heterohybridoma production. Phage display technology, by contrast, relies on molecular
30 techniques for potentially capturing the entire repertoire of immunoglobulin genes associated with the response to a given antigen.

-16-

This technique is described by Barbas et al, Proc. Natl. Acad. Sci., USA, 88, 7978-7982, (1991).

Essentially, immunoglobulin heavy chain genes are PCR amplified and cloned into a vector containing the gene
5 ~~encoding the minor coat protein of the filamentous phage~~
M13 in such a way that a heavy chain fusion protein is created. The heavy chain fusion protein is incorporated into the M13 phage particle together with the light
chain genes as it assembles. Each recombinant phage
10 contains, within its genome, the genes for a different antibody Fab molecule which it displays on its surface. Within these libraries, in excess of 10^6 different antibodies can be cloned and displayed. The phage library is panned on antigen coated microliter wells,
15 non-specific phage are washed off, and antigen binding phage are eluted. The genome from the antigen-specific clones is isolated and the gene III is excised, so that antibody can be expressed in soluble Fab form for further characterization. Once a single Fab is selected
20 as a potential therapeutic candidate, it may easily be converted to a whole antibody. A previously described expression system for converting Fab sequences to whole antibodies is IDEC's mammalian expression vector NEOSPLA. This vector contains either human gamma 1 or
25 gamma 4 constant region genes. CHO cells are transfected with the NEOSPLA vectors and after amplification this vector system has been reported to provide very high expression levels (> 30 pg/cell/day) can be achieved.

30 4. Primatized Antibodies

Another highly efficient means for generating recombinant antibodies is disclosed by Newman, (1992), Biotechnology, 10, 1455-1460. More particularly, this

-17-

technique results in the generation of primatized antibodies which contain monkey variable domains and human constant sequences. This reference is incorporated by reference in its entirety herein.

5 ~~Moreover, this technique is also described in commonly~~
assigned U.S. Application No. 08/379,072, filed on
January 25, 1995, which is a continuation of U.S. Serial
No. 07/912,292, filed July 10, 1992, which is a
continuation-in-part of U.S. Serial No. 07/856,281,
10 filed March 23, 1992, which is finally a continuation-
in-part of U.S. Serial No. 07/735,064, filed July 25,
1991. 08/379,072 and the parent application thereof are
incorporated by reference in their entirety herein.

This technique modifies antibodies such that they
15 are not antigenically rejected upon administration in
humans. This technique relies on immunization of
cynomolgus monkeys with human antigens or receptors.
This technique was developed to create high affinity
monoclonal antibodies directed to human cell surface
20 antigens.

Identification of macaque antibodies to human B7.1
and B7.2 by screening of phage display libraries or
monkey heterohybridomas obtained using B lymphocytes
from B7.1 and/or B7.2 immunized monkeys is also
25 described in commonly assigned U.S. Application No.
08/487,550, filed June 7, 1995, incorporated by
reference in its entirety herein. More specifically,
08/487,550 provides four monoclonal antibodies 7B6,
16C10, 7C10 and 20C9 which inhibit the B7:CD28 pathway
30 and thereby function as effective immunosuppressants.

Antibodies generated in the manner described by
these co-assigned applications have previously been
reported to display human effector function, have
reduced immunogenicity, and long serum half-life. The

-18-

technology relies on the fact that despite the fact that cynomolgus monkeys are phylogenetically similar to humans, they still recognize many human proteins as foreign and therefore mount an immune response.

5 — Moreover, because the cynomolgus monkeys are phylogenetically close to humans, the antibodies generated in these monkeys have been discovered to have a high degree of amino acid homology to those produced in humans. Indeed, after sequencing macaque
10 immunoglobulin light and heavy chain variable region genes, it was found that the sequence of each gene family was 85-98% homologous to its human counterpart (Newman et al, (1992), Id.). The first antibody generated in this way, an anti-CD4 antibody, was 91-92%
15 homologous to the consensus sequence of human immunoglobulin framework regions. Newman et al, Biotechnology, 10:1458-1460, (1992).

Monoclonal antibodies specific to the human B7 antigen have been previously described in the
20 literature. For example, Weyl et al, Hum. Immunol., 31(4), 271-276, (1991) describe epitope mapping of human monoclonal antibodies against HLA-B-27 using natural and mutated antigenic variants. Also, Toubert et al, Clin. Exp. Immunol., 82(1), 16-20, (1990) describe epitope
25 mapping of an HLA-B27 monoclonal antibody that also reacts with a 35-KD bacterial outer membrane protein. Also, Valle et al, Immunol., 69(4), 531-535, (1990) describe a monoclonal antibody of the IgG1 subclass which recognizes the B7 antigen expressed in activated B
30 cells and HTLV-1-transformed T cells. Further, Toubert et al, J. Immunol., 141(7), 2503-9, (1988) describe epitope mapping of HLA-B27 and HLA-B7 antigens using intradomain recombinants constructed by making hybrid genes between these two alleles in *E. coli*.

-19-

High expression of B7 antigen has been correlated to autoimmune diseases by some researchers. For example, Ionesco-Tirgoviste et al, Med. Interre, 24(1), 11-17, (1986) report increased B7 antigen expression in type 1 insulin-dependent diabetes. Also, the involvement of B7 antigen expression on dermal dendritic cells obtained from psoriasis patients has been reported. (Nestle et al, J. Clin. Invest., 94(1), 202-209, (1994)).

Further, the inhibition of anti-HLA-B7 alloreactive CTL using affinity-purified soluble HLA-B7 has been reported in the literature. (Zavazava et al, Transplantation, 51(4), 838-42, (1991)). Further, the use of B7 receptor soluble ligand, CTLA-4-Ig to block B7 activity (See, e.g., Lenschow et al, Science, 257, 789, 7955 (1992)) in animal models and a B7.1-Ig fusion protein capable of inhibiting B7 has been reported.

Evidence is provided in this disclosure for the identification of monoclonal antibodies which recognize specific sites on the B7.1 antigen which are restricted to CD28 receptor binding. Furthermore, evidence is presented herein for the identification of antibodies which recognize sites on the B7.1 antigen which are exclusive of CTLA-4 receptor binding. Thus, evidence is presented herein to support the existence of unique antigen binding sites on the human B7.1 (CD80) co-stimulatory antigen. The sites claimed are identified by anti-B7.1 PRIMATIZED® antibodies and evidence is presented which confirms binding to a site of interaction on the B7.1 antigen which is restricted to binding with the co-activation receptor CD28.

SUMMARY AND OBJECTS OF THE INVENTION

-20-

An object of the invention is to identify novel antibodies which are specific to human B7.1 antigen. More specifically, it is an object of the invention to identify antibodies which are specific to human B7.1 antigen and which are also capable of inhibiting the binding of B7.1 to a CD28 receptor. It is also an object of this invention to identify antibodies which are specific to human B7.1 antigen and which are not capable of inhibiting the binding of B7.1 to a CTLA-4 receptor. Thus, an object of this invention is to identify antigens which recognize specific sites on the B7.1 antigen, wherein the recognized sites are restricted to CD28 receptor binding and which are exclusive of CTLA-4 receptor binding.

It is a further object of the invention to identify antibodies which are specific to human B7.1 antigen and which fail to recognize human B7.2 antigen.

It is another object of the invention to identify monoclonal antibodies and primatized forms thereof which recognize specific sites on the human B7.1 antigen and which inhibit IL-2 production and T cell proliferation and which function as effective immunosuppressants. More specifically, it is an object of this invention to identify antibodies which are specific to B7.1 and which are capable of inhibiting IL-2 production.

It is another object of the invention to provide monoclonal antibodies and primatized forms thereof which inhibit antigen driven responses in donor spleen cell cultures, e.g., antigen specific IgG responses, IL-2 production and cell proliferation.

It is another specific object of the invention to identify particular monoclonal antibodies specific to human B7.1 antigen and primatized forms thereof having advantageous properties, i.e., affinity,

-21-

immunosuppressive activity, which are useful as therapeutics. More specifically, these antibodies and primatized forms thereof are to be used, e.g., as immunosuppressants, i.e., to block antigen driven immune

5 ~~responses, to treat autoimmune diseases such as~~
psoriasis, rheumatoid arthritis, systemic erythematosus (SLE), type 1 diabetes mellitus, idiopathic thrombocytopenia purpura (ITP), allergy, inflammatory bile disease, and to prevent organ rejection.

10 It is another object of the invention to provide pharmaceutical compositions containing one or more monoclonal antibodies specific to human B7.1 antigen or primatized forms thereof, and a pharmaceutically acceptable carrier or excipient. These compositions
15 will be used, e.g., as immunosuppressants to treat autoimmune diseases, e.g., idiopathic thrombocytopenia purpura (ITP) and systemic lupus erythematosus (SLE), to block antigen driven immune responses, and to prevent organ rejection in transplant recipients.

20 It is another object of the invention to provide novel methods of therapy by administration of therapeutically effective amounts of one or more or primatized monoclonal antibodies which specifically bind to human B7.1 antigen. Such therapeutic methods are
25 useful for treatment of diseases treatable by inhibition of the B7:CD28 pathway, e.g., autoimmune diseases such as idiopathic thrombocytopenia purpura (ITP), systemic lupus erythematosus (SLE), type 1 diabetes mellitus, psoriasis, rheumatoid arthritis, multiple sclerosis,
30 aplastic anemia, as well as for preventing rejection in transplantation subjects.

It is still another object of the invention to provide transfectants, e.g., CHO cells, which express at

-22-

least the variable heavy and light domains of monoclonal antibodies specific to the human B7.1 antigen.

Definitions

The following terms are defined so that the

5 invention may be more clearly understood.

Depleting antibody - an antibody which kills activated B cells or other antigen presenting cells.

Non-depleting antibody - an antibody which blocks the co-stimulatory action of B7 and T cell activating
10 ligands CD28 and CTLA-4. Thus, it anergizes but does not eliminate the antigen presenting cell.

Primatized antibody - a recombinant antibody which has been engineered to contain the variable heavy and light domains of a monkey antibody, in particular, a
15 cynomolgus monkey antibody, and which contains human constant domain sequences, preferably the human immunoglobulin gamma 1 or gamma 4 constant domain (or PE variant). The preparation of such antibodies is described in Newman et al, (1992), "Primatization of
20 Recombinant Antibodies for Immunotherapy of Human Diseases: A Macaque/Human Chimeric Antibody Against Human CDH, Biotechnology, 10:1458-1460; also in commonly assigned 08/379,072 both of which are incorporated by reference in their entirety herein. These antibodies
25 have been reported to exhibit a high degree of homology to human antibodies, i.e., 85-98%, display human effector functions, have reduced immunogenicity, and may exhibit high affinity to human antigens.

B7 antigens - B7 antigens in this application include,
30 e.g., human B7, B7.1 and B7.2 antigens. These antigens bind to CD28 and/or CTLA-4. These antigens have a co-stimulatory role in T cell activation. Also, these B7 antigens all contain extracellular immunoglobulin

-23-

superfamily V and C-like domains, a hydrophobic transmembrane region and a cytoplasmic tail. (See, Freeman et al, Science, 262:909, (1993)), and are heavily glycosylated.

- 5 Anti-B7 antibodies - Antibodies, preferably monkey monoclonal antibodies or primatized forms thereof, which specifically bind human B7 antigens, e.g., human B7.1 and/or B7.2 antigen with a sufficient affinity to block the B7:CD28 interaction and thereby induce
10 immunosuppression.

BRIEF DESCRIPTION OF THE FIGURES

- Figure 1 depicts the pMS vector used to screen recombinant immunoglobulin libraries produced against B7 displayed on the surface of filamentous phage which
15 contains primers based on macaque immunoglobulin sequences.

Figure 2 depicts the NEOSPLA expression vector used to express the subject primatized antibodies specific to human B7.1 antigen.

- 20 Figure 3a depicts the amino acid and nucleic acid sequence of a primatized form of the light chain of 7C10.

- Figure 3b depicts the amino acid and nucleic acid sequence of a primatized form of the heavy chain of
25 7C10.

Figure 4a depicts the amino acid and nucleic acid sequence of a primatized form of the light chain of 7B6.

Figure 4b depicts the amino acid and nucleic acid sequence of a primatized form of the heavy chain of 7B6.

- 30 Figure 5a depicts the amino acid and nucleic acid sequence of a primatized light chain 16C10.

Figure 5b depicts the amino acid and nucleic acid sequence of a primatized heavy chain 16C10.

-24-

Figure 6 depicts the inability of P16C10 to block CTLA-4Ig-Biotin binding to B7.1 transfected CHO cells.

Figure 7 depicts the inability of CTLA-4Ig to block P16C10-Biotin binding to B7.1 transfected CHO cells.

5 ~~Figure 8 depicts that BB-1 completely blocks~~
binding of CTLA-4Ig-Biotin to B7.1 transfected CHO cells
and further depicts the inability of BB-1 to
significantly affect P16C10-Biotin binding to B7.1
transfected CHO cells.

10 Figure 9 depicts that CTLA-4Ig-Biotin is
effectively blocked by all B7.1 inhibitors except
P16C10.

Figure 10 depicts the ability of P16C10 to block
binding of the CD28/B7-1Ig interaction. Data shown are
15 averages of values obtained from four separate
experiments.

DETAILED DESCRIPTION OF THE INVENTION

As described above, the present invention relates
to the identification of monoclonal antibodies or
20 primatized forms thereof which are specific to human
B7.1 antigen and which are capable of inhibiting the
binding of B7.1 to a CD28 receptor and which are not
capable of inhibiting the binding of B7.1 to a CTLA-4
receptor. Blocking of the primary activation site
25 between CD28 and B7.1 (CD80) with the identified
antibodies while allowing the combined antagonistic
effect on positive co-stimulation with an agnostic
effect on negative signaling will be a useful
therapeutic approach for intervening in relapsed forms
30 of autoimmune disease. The functional activity of the
identified antibodies is defined by blocking the
production of the T cell stimulatory cytokine IL-2.
Identified antibodies have demonstrated the ability to

-25-

block the production of IL-2 in excess of 50%, in spite of the existence of a second actuating ligand B7.2, suggesting an alternate mechanism of action exists which is not typical of the observed effects of other anti-
5 B7.1 antibodies defined in the literature.

Manufacture of novel monkey monoclonal antibodies which specifically bind human B7.1 and/or human B7.2 antigen, as well as primatized antibodies derived therefrom is described in co-pending U.S. Application
10 Serial No. 08/487,550, and as set forth herein. These antibodies possess high affinity to human B7.1 and/or B7.2 and therefore may be used as immunosuppressants which inhibit the B7:CD86 pathway.

Preparation of monkey monoclonal antibodies will
15 preferably be effected by screening of phage display libraries or by preparation of monkey heterohybridomas using B lymphocytes obtained from B7 (e.g., human B7.1 and/or B7.2) immunized monkeys.

As noted, the first method for generating anti-B7
20 antibodies involves recombinant phage display technology. This technique is generally described *supra*.

Essentially, this will comprise synthesis of recombinant immunoglobulin libraries against B7 antigen
25 displayed on the surface of filamentous phage and selection of phage which secrete antibodies having high affinity to B7.1 and/or B7.2 antigen. As noted *supra*, preferably antibodies will be selected which bind to both human B7.1 and B7.2. To effect such methodology,
30 the present inventors have created a unique library for monkey libraries which reduces the possibility of recombination and improves stability. This vector, PMS, is described in detail *infra*, and is shown in Figure 1.

-26-

Essentially, to adopt phage display for use with macaque libraries, this vector contains specific primers for PCR amplifying monkey immunoglobulin genes. These primers are based on macaque sequences obtained while
5 developing the primatized technology and databases containing human sequences.

Suitable primers are disclosed in commonly assigned 08/379,072 incorporated by reference herein.

The second method involves the immunization of
10 monkeys, i.e., macaques, against human B7 antigen, preferably against human B7.1 and B7.2 antigen. The inherent advantage of macaques for generation of monoclonal antibodies is discussed *supra*. In particular, such monkeys, i.e., cynomolgus monkeys, may
15 be immunized against human antigens or receptors. Moreover, the resultant antibodies may be used to make primatized antibodies according to the methodology of Newman et al, Biotechnology, 10, 1455-1460, (1992), and Newman et al, commonly assigned U.S. Serial No.
20 08/379,072, filed January 25, 1995, which are incorporated by reference in their entirety.

The significant advantage of antibodies obtained from cynomolgus monkeys is that these monkeys recognize many human proteins as foreign and thereby provide for
25 the formation of antibodies, some with high affinity to desired human antigens, e.g., human surface proteins and cell receptors. Moreover, because they are phylogenetically close to humans, the resultant antibodies exhibit a high degree of amino acid homology to those
30 produced in humans. As noted above, after sequencing macaque immunoglobulin light and heavy variable region genes, it was found that the sequence of each gene family was 85-88% homologous to its human counterpart (Newman et al, (1992), Id.).

-27-

Essentially, cynomolgus macaque monkeys are administered human B7 antigen, e.g., human B7.1 and/or human B7.2 antigen, B cells are isolated therefrom, e.g., lymph node biopsies are taken from the animals, and B-lymphocytes are then fused with KH6/B5 (mouse x human) heteromyeloma cells using polyethylene glycol (PEG). Heterohybridomas secreting antibodies which bind human B7 antigen, e.g., human B7.1 and/or human B7.2 antigen, are then identified.

10 Antibodies which bind to both B7.1 and B7.2 are desirable because such antibodies potentially may be used to inhibit the interaction of B7.1 and B7.2, as well as B7 with their counter-receptors, i.e., human CTLA-4 and CD28. Antibodies against these epitopes may
15 inhibit the interaction of both human B7.1 and human B7.2 with their counter receptors on the T cell. This may potentially provide synergistic effects.

 However, antibodies which bind to only one of human B7 antigen, B7.1 antigen or B7.2 antigen, are also
20 highly desirable because of the co-involvement of these molecules in T cell activation, clonal expansion lymphokine (IL-2) secretion, and responsiveness to antigen. Given that both human B7.1 and B7.2 bind to human CTLA-4 and CD28, it is probable that there is at
25 least one common or homologous region (perhaps a shared conformational epitope or epitopes) to which macaque antibodies may potentially be raised.

 The disclosed invention involves the use of an animal which is primed to produce a particular antibody.
30 Animals which are useful for such a process include, but are not limited to, the following: mice, rats, guinea pigs, hamsters, monkeys, pigs, goats and rabbits.

-28-

A preferred means of generating human antibodies using SCID mice is disclosed in commonly-owned, co-pending U.S. Patent application Serial No. 08/488,376.

The present inventors elected to immunize macaques
5 against human B7.1 antigen using recombinant soluble
B7.1 antigen produced in CHO cells and purified by
affinity chromatography using a L307.4-sepharose
affinity column. However, the particular source of
human B7 antigen, human B7.1 antigen or human B7.2
10 antigen is not critical, provided that it is of
sufficient purity to result in a specific antibody
response to the particular administered B7 antigen and
potentially to other B7 antigens.

The human B7 antigen, human B7.1 antigen (also
15 called CD80) and human B7.2 antigen (also called CD86)
genes have been cloned, and sequenced, and therefore may
readily be manufactured by recombinant methods.

Preferably, the administered human B7 antigen,
human B7.1 antigen and/or human B7.2 antigen will be
20 administered in soluble form, e.g., by expression of a
B7, B7.1 or B7.2 gene which has its transmembrane and
cytoplasmic domains removed, thereby leaving only the
extracellular portion, i.e., the extracellular
superfamily V and C-like domains. (See, e.g., Grumet et
25 al, Hum. Immunol., 40(3), p. 228-234, 1994, which
teaches expression of a soluble form of human B7, which
is incorporated by reference in its entirety herein).

The macaques will be immunized with the B7, B7.1
and/or B7.2 antigen, preferably a soluble form thereof,
30 under conditions which result in the production of
antibodies specific thereto. Preferably, the soluble
human B7, B7.1 or B7.2 antigen will be administered in
combination with an adjuvant, e.g., Complete Freund's
Adjuvant (CFA), Alum, Saponin, or other known adjuvants,

-29-

as well as combinations thereof. In general, this will require repeated immunization, e.g., by repeated injection, over several months. For example, administration of soluble B7.1 antigen was effected in
5 adjuvant, with booster immunizations, over a 3 to 4 month period, with resultant production of serum containing antibodies which bound human B7.1 antigen.

After immunization B cells are collected, e.g., by lymph node biopsies taken from the immunized animals and
10 B lymphocytes fused with KH6/B5 (mouse x human) heteromyeloma cells using polyethylene glycol. Methods for preparation of such heteromyelomas are known and may be found in U.S. Serial No. 08/379,072 by Newman et al, filed on January 25, 1995 and incorporated by reference
15 herein.

Heterohybridomas which secrete antibodies which bind human B7, B7.1 and/or B7.2 are then identified. This may be effected by known techniques. For example, this may be determined by ELISA or radioimmunoassay
20 using enzyme or radionucleotide labelled human B7, B7.1 and/or B7.2 antigen.

Cell lines which secrete antibodies having the desired specificity to human B7, B7.1 and/or B7.2 antigen are then subcloned to monoclonality.

25 In the present invention, the inventors screened purified antibodies for their ability to bind to soluble B7.1 antigen coated plates in an ELISA assay, antigen positive B cells, and CHO transfectomas which express human B7.1 antigen on their cell surface. In addition,
30 the antibodies were screened for their ability to block B cell/T cell interactions as measured by IL-2 production and tritiated thymidine uptake in a mixed lymphocyte reaction (MLR), with B7 binding being detected using ¹²⁵I-radiolabeled soluble B7.1 (SB7.1).

-30-

Also, affinity purified antibodies from macaques were tested for their reactivity against CHO transfectants which expressed B7.1/Ig fusion proteins, and against CHO cells which produced human B7.2 antigen.

5 ~~These results indicated that the B7.1 immune sera bound~~
to the B7.2 transfectomas. Binding of antibodies to B7.2 antigen may be confirmed using soluble B7.2-Ig reagents. As discussed in the examples, this may be effected by producing and purifying B7.2-Ig from CHO
10 transfectomas in sufficient quantities to prepare a B7.2-Ig-sepharose affinity column. Those antibodies which cross-react with B7.2 will bind the B7.2-Ig-sepharose column.

Cell lines which express antibodies which
15 specifically bind to human B7 antigen, B7.1 antigen and/or B7.2 antigen are then used to clone variable domain sequences for the manufacture of primatized antibodies essentially as described in Newman et al, (1992), Id. and Newman et al, U.S. Serial No. 379,072,
20 filed January 25, 1995, both of which are incorporated by reference herein. Essentially, this entails extraction of RNA therefrom, conversion to cDNA, and amplification thereof by PCR using Ig specific primers. Suitable primers are described in Newman et al, 1992,
25 Id. and in U.S. Serial No. 379,072. (See, in particular, Figure 1 of U.S. Serial No. 379,072).

The cloned monkey variable genes are then inserted into an expression vector which contains human heavy and light chain constant region genes. Preferably, this is
30 effected using a proprietary expression vector of IDEC, Inc., referred to as NEOSPLA. This vector is shown in Figure 2 and contains the cytomegalovirus promoter/enhancer, the mouse beta globin major promoter, the SV40 origin of replication, the bovine growth

-31-

hormone polyadenylation sequence, neomycin phosphotransferase exon 1 and exon 2, human immunoglobulin kappa or lambda constant region, the dihydrofolate reductase gene, the human immunoglobulin gamma 1 or gamma 4 PE constant region and leader sequence. This vector has been found to result in very high level expression of primatized antibodies upon incorporation of monkey variable region genes, transfection in CHO cells, followed by selection in G418 containing medium and methotrexate amplification.

For example, this expression system has been previously disclosed to result in primatized antibodies having high avidity ($K_d \leq 10^{-10}$ M) against CD4 and other human cell surface receptors. Moreover, the antibodies have been found to exhibit the same affinity, specificity and functional activity as the original monkey antibody. This vector system is substantially disclosed in commonly assigned U.S. Serial No. 379,072, incorporated by reference herein as well as U.S. Serial No. 08/149,099, filed on November 3, 1993, also incorporated by reference in its entirety herein. This system provides for high expression levels, i.e., > 30 pg/cell/day.

As discussed *infra*, the subject inventors have selected four lead candidate monkey monoclonal antibodies which specifically bind the B7.1 antigen. These monkey monoclonal antibodies are referred to herein as 7B6, 16C10, 7C10 and 20C9.

As discussed in greater detail *infra*, these antibodies were evaluated for their ability to block B cell/T cell interactions as measured by IL-2 production and tritiated thymidine uptake in a mixed lymphocyte reaction for T cell binding experiments for T cell binding, human buffy coat peripheral blood lymphocytes

-32-

were cultured for 3-6 days in the presence of PHA stimulator. B7 binding was radioassayed using ^{125}I -radiolabeled soluble B7.1. The observed results indicate that all of these antibodies bind B7.1 antigen

- 5 with high affinity and effectively block B cell/T cell interactions as evidenced by reduced IL-2 production and reduced proliferation of mixed lymphocyte cultures.

The properties of these particular monkey monoclonal antibodies are summarized below:

- 10 1. Scatchard analysis showed that the apparent affinity constants (K_d) for the monkey antibodies binding to B7-Ig coated plates were approximated to be:
- 15 a: 7C10: $6.2 \times 10^{-9}\text{M}$
b: 16C10: $8.1 \times 10^{-9}\text{M}$
c: 7B6: $10.7 \times 10^{-9}\text{M}$
d: 20C9: $16.8 \times 10^{-9}\text{M}$
- 20 2. The antibodies were tested *in vitro* in a mixed lymphocyte reaction assay (MLR). The MLR showed that all 4 anti-B7.1 antibodies inhibit IL-2 production to different extents as shown by the following IC_{50} values:
- 25 a: 7B6: $5.0 \mu\text{g/M}$
b: 16C10: $<0.1 \mu\text{g/M}$
c: 20C9: $2.0 \mu\text{g/M}$
d: 7C10: $5.0 \mu\text{g/M}$
- 30 3. The monkey anti-B7.1 antibodies were tested for their ability to bind B7 on human peripheral blood lymphocytes (PBL). FACS analysis showed that all 4 monkey antibodies tested positive.
- 35 4. Monkey antibodies 16C10, 7B6, 7C10 and 20C9 were tested for C1q binding by FACS analysis. Results showed 7C10 monkey Ig had strong human C1q binding after incubating with B7.1 CHO-

-33-

transfected cells. 16C10 was positive, while 20C9 and 7B6 monkey antibodies were negative.

5. To select an animal model for path-tox studies, the monkey antibodies were tested with animal blood from different species. It was determined that the monkey anti-B7.1 antibodies cross-reacted with human, chimpanzee.

Based on these properties, it would appear that three monkey monoclonal antibodies possess the most advantageous properties, 16C10, 7C10 and 20C9, with 16C10 and 7C10 being somewhat better than 20C9.

Using the techniques described *supra*, and in commonly assigned U.S. Serial No. 08/379,072, the present inventors have cloned the variable domains of 7C10, 7B6 and 16C10, and provide the amino acid and nucleic acid sequences of primatized forms of the 7C10 light chain, 7C10 heavy chain, 7B6 light chain, 7B6 heavy chain, 16C10 light chain and 16C10 heavy chain. These amino acid and nucleic acid sequences may be found in Figures 3a and 3b, 4a and 4b, and 5a and 5b. The DNA and amino acid sequence for the human gamma 1, gamma 4 constant domain may be found in 08/379,072.

As discussed *supra*, these primatized antibodies are preferably expressed using the NEOSPLA expression vector shown in Figure 2 which is substantially described in commonly assigned 08/379,072 and 08/149,099, both of which applications are incorporated by reference herein.

As previously noted, the subject primatized antibodies will preferably contain either the human immunoglobulin gamma 1 or gamma 4 constant region, with gamma 4 preferably mutated at two positions to create gamma 4 PE. The gamma 4 PE mutant contains two mutations, a glutamic acid in the CH2 region introduced

-34-

to eliminate residual FCR binding, and a proline substitution in the hinge region, intended to enhance the stability of the heavy chain disulfide bond interaction. (See, Alegre et al, J. Immunol., 148, 3461-3468, (1992); and Angel et al, Mol. Immunol., 30, 105-158, (1993), both of which are incorporated by reference herein).

Whether the subject primatized antibodies contain the gamma 1, gamma 4 or gamma 4 PE constant region largely depends on the particular disease target. Preferably, depleting and non-depleting primatized IgG1 and IgG4 antibodies are created and tested against specific disease targets.

Given the described binding and functional properties of the subject monkey monoclonal antibodies, these anti-B7.1 monoclonal antibodies and primatized forms thereof should be well suited as therapeutic agents for blocking the B7:CD28 interaction thereby providing for immunosuppression. In particular, given their high affinity to B7.1 antigen and ability to block B cell/T cell interactions as measured by IL-2 production and tritiated thymidine uptake in mixed lymphocyte culture as well as their ability to effectively inhibit antigen driven responses in donor spleen cell cultures as shown by reduced antigen specific IgG responses, IL-2 production and cell proliferation, these monkey monoclonal antibodies and primatized forms thereof should function as effective immunosuppressants which modulate the B7:CD28 pathway. This is significant for the treatment of many diseases wherein immunosuppression is therapeutically desirable, e.g., autoimmune diseases, to inhibit undesirable antigen specific IgG responses, and also for prevention of organ rejection and graft-versus-host disease.

-35-

Essentially, the subject antibodies will be useful in treating any disease wherein suppression of the B7:CD28 pathway is therapeutically desirable.

Key therapeutic indications for the subject anti-B7.1 antibodies include, by way of example, autoimmune diseases such as idiopathic thrombocytopenia purpura (ITP), systemic lupus erythematosus (SLE), type 1 diabetes mellitus, multiple sclerosis, aplastic anemia, psoriasis, allergy, inflammatory bile disease and rheumatoid arthritis.

Another significant therapeutic indication of the subject anti-B7.1 antibodies is for prevention of graft-versus-host-disease (GVHD) during organ transplant and bone marrow transplant (BMT). The subject antibodies may be used to induce host tolerance to donor-specific alloantigens and thereby facilitate engraftment and reduce the incidence of graft rejection. It has been shown in a murine model of allogeneic cardiac transplantation that intravenous administration of CTLA4-Ig can result in immunosuppression or even induction of tolerance to alloantigen. (Lin et al, J. Exp. Med. 178:1801, 1993; Torka et al, Proc. Natl. Acad. Sci., USA, 89:11102, 1992). It is expected that the subject primatized anti-B7.1 antibodies will exhibit similar or greater activity.

Antibodies produced in the manner described above, or by equivalent techniques, can be purified by a combination of affinity and size exclusion chromatography for characterization in functional biological assays. These assays include determination of specificity and binding affinity as well as effector function associated with the expressed isotype, e.g., ADCC, or complement fixation. Such antibodies may be used as passive or active therapeutic agents against a

-36-

number of human diseases, including B cell lymphoma, infectious diseases including viral diseases such as HIV/AIDS, autoimmune and inflammatory diseases, and transplantation. The antibodies can be used either in
5 their native form, or as part of an antibody/chelate, antibody/drug or antibody/toxin complex. Additionally, whole antibodies or antibody fragments (Fab₂, Fab, Fv) may be used as imaging reagents or as potential vaccines or immunogens in active immunotherapy for the generation
10 of anti-idiotypic responses.

The amount of antibody useful to produce a therapeutic effect can be determined by standard techniques well known to those of ordinary skill in the art. The antibodies will generally be provided by
15 standard technique within a pharmaceutically acceptable buffer, and may be administered by any desired route. Because of the efficacy of the presently claimed antibodies and their tolerance by humans it is possible to administer these antibodies repetitively in order to
20 combat various diseases or disease states within a human.

The anti-B7.1 antibodies (or fragments thereof) of this invention are useful for inducing immunosuppression, i.e., inducing a suppression of a
25 human's or animal's immune system. This invention therefore relates to a method of prophylactically or therapeutically inducing immunosuppression in a human or other animal in need thereof by administering an effective, non-toxic amount of such an antibody of this
30 invention to such human or other animal.

The ability of the compounds of this invention to induce immunosuppression has been demonstrated in standard tests used for this purpose, for example, a mixed lymphocyte reaction test or a test measuring

-37-

inhibition of T-cell proliferation measured by thymidine uptake.

The fact that the antibodies of this invention have utility in inducing immunosuppression indicates that

5 they should be useful in the treatment or prevention of resistance to or rejection of transplanted organs or tissues (e.g., kidney, heart, lung, bone marrow, skin, cornea, etc.); the treatment or prevention of

10 autoimmune, inflammatory, proliferative and hyperproliferative diseases, and of cutaneous manifestations of immunologically mediated diseases (e.g., rheumatoid arthritis, lupus erythematosus, systemic lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type 1 diabetes,

15 uveitis, nephrotic syndrome, psoriasis, atopic dermatitis, contact dermatitis and further eczematous dermatitides, seborrheic dermatitis, Lichen planus, Pemphigus, bullous pemphigus, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythema,

20 cutaneous eosinophilias, Alopecia areata, etc.); the treatment of reversible obstructive airways disease, intestinal inflammations and allergies (e.g., inflammatory bile disease, Coeliac disease, proctitis, eosinophilia gastroenteritis, mastocytosis, Crohn's

25 disease and ulcerative colitis), food-related allergies (e.g., migraine, rhinitis and eczema), and other types of allergies.

One skilled in the art would be able, by routine experimentation, to determine what an effective, non-

30 toxic amount of antibody would be for the purpose of inducing immunosuppression. Generally, however, an effective dosage will be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

-38-

The antibodies (or fragments thereof) of this invention should also be useful for treating tumors in a mammal. More specifically, they should be useful for reducing tumor size, inhibiting tumor growth and/or

5 prolonging the survival time of tumor-bearing animals. Accordingly, this invention also relates to a method of treating tumors in a human or other animal by administering to such human or animal an effective, non-toxic amount of an antibody. One skilled in the art

10 would be able, by routine experimentation, to determine what an effective, non-toxic amount of anti-B7 antibody would be for the purpose of treating carcinogenic tumors. Generally, however, an effective dosage is expected to be in the range of about 0.05 to 100

15 milligrams per kilogram body weight per day.

The antibodies of the invention may be administered to a human or other animal in accordance with the aforementioned methods of treatment in an amount sufficient to produce such effect to a therapeutic or

20 prophylactic degree. Such antibodies of the invention can be administered to such human or other animal in a conventional dosage form prepared by combining the antibody of the invention with a conventional pharmaceutically acceptable carrier or diluent according

25 to known techniques. It will be recognized by one of skill in the art that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and

30 other well-known variables.

The route of administration of the antibody (or fragment thereof) of the invention may be oral, parenteral, by inhalation or topical. The term parenteral as used herein includes intravenous,

-39-

intraperitoneal, intramuscular, subcutaneous, rectal or vaginal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred.

5 The daily parenteral and oral dosage regimens for
employing compounds of the invention to prophylactically
or therapeutically induce immunosuppression, or to
therapeutically treat carcinogenic tumors will generally
10 be in the range of about 0.05 to 100, but preferably
about 0.5 to 10, milligrams per kilogram body weight per
day.

 The antibodies of the invention may also be
administered by inhalation. By "inhalation" is meant
intranasal and oral inhalation administration.
15 Appropriate dosage forms for such administration, such
as an aerosol formulation or a metered dose inhaler, may
be prepared by conventional techniques. The preferred
dosage amount of a compound of the invention to be
employed is generally within the range of about 10 to
20 100 milligrams.

 The antibodies of the invention may also be
administered topically. By topical administration is
meant non-systemic administration and includes the
application of an antibody (or fragment thereof)
25 compound of the invention externally to the epidermis,
to the buccal cavity and instillation of such an
antibody into the ear, eye and nose, and where it does
not significantly enter the blood stream. By systemic
administration is meant oral, intravenous,
30 intraperitoneal and intramuscular administration. The
amount of an antibody required for therapeutic or
prophylactic effect will, of course, vary with the
antibody chosen, the nature and severity of the
condition being treated and the animal undergoing

-40-

treatment, and is ultimately at the discretion of the physician. A suitable topical dose of an antibody of the invention will generally be within the range of about 1 to 100 milligrams per kilogram body weight daily.

Formulations

While it is possible for an antibody or fragment thereof to be administered alone, it is preferable to present it as a pharmaceutical formulation. The active ingredient may comprise, for topical administration, from 0.001% to 10% w/w, e.g., from 1% to 2% by weight of the formulation, although it may comprise as much as 10% w/w but preferably not in excess of 5% w/w and more preferably from 0.1% to 1% w/w of the formulation.

The topical formulations of the present invention, comprise an active ingredient together with one or more acceptable carrier(s) therefor and optionally any other therapeutic ingredients(s). The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a

-41-

surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 90°-100°C for half an hour. Alternatively, the solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its

-42-

derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogols. The formulation may incorporate any suitable surface active agent such as an anionic, 5 cationic or non-ionic surface active such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous silicas, and other ingredients such as lanolin, may also 10 be included.

The subject anti-B7.1 antibodies or fragments thereof may also be administered in combination with other moieties which modulate the B7:CD28 pathway. Such moieties include, by way of example, cytokines such as 15 IL-7 and IL-10, CTLA4-Ig, soluble CTLA-4 and anti-CD28 antibodies and fragments thereof. Also, the subject antibodies may be administered in combination with other immunosuppressants. Such immunosuppressants include small molecules such as cyclosporin A (CSA) and FK506; 20 monoclonal antibodies such as anti-tumor necrosis factor α (anti-TNF α), anti-CD54, anti-CD11, anti-CD11a, and anti-IL-1; and, other soluble receptors such as rTNF α and rIL-1.

It will be recognized by one of skill in the art 25 that the optimal quantity and spacing of individual dosages of an antibody or fragment thereof of the invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular animal being treated, 30 and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses of an antibody or fragment thereof of the invention given per day for a defined number of

-43-

days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

Without further elaboration, it is believed that
5 one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following formulations are, therefore, to be construed as merely illustrative embodiments and not a limitation of the scope of the
10 present invention in any way.

Capsule Composition

A pharmaceutical composition of this invention in the form of a capsule is prepared by filling a standard two-piece hard gelatin capsule with 50 mg. of an
15 antibody or fragment thereof of the invention, in powdered form, 100 mg. of lactose, 32 mg. of talc and 8 mg. of magnesium stearate.

Injectable Parenteral Composition

A pharmaceutical composition of this invention in a
20 form suitable for administration by injection is prepared by stirring 1.5% by weight of an antibody or fragment thereof of the invention in 10% by volume propylene glycol and water. The solution is sterilized by filtration.

Ointment Composition

Antibody or fragment thereof of the invention
1.0 g.

White soft paraffin to 100.0 g.

The antibody or fragment thereof of the invention
30 is dispersed in a small volume of the vehicle to produce

-44-

a smooth, homogeneous product. Collapsible metal tubes are then filled with the dispersion.

Topical Cream Composition

Antibody or fragment thereof of the invention

5 1.0 g.

Polawax GP 200 20.0 g.

Lanolin Anhydrous 2.0 g.

White Beeswax 2.5 g.

Methyl hydroxybenzoate 0.1 g.

10 Distilled Water to 100.0 g.

The polawax, beeswax and lanolin are heated together at 60°C. A solution of methyl hydroxybenzoate is added and homogenization is achieved using high speed stirring. The temperature is then allowed to fall to
15 50°C. The antibody or fragment thereof of the invention is then added and dispersed throughout, and the composition is allowed to cool with slow speed stirring.

Topical Lotion Composition

Antibody or fragment thereof of the invention

20 1.0 g.

Sorbitan Monolaurate 0.6 g.

Polysorbate 20 0.6 g.

Cetostearyl Alcohol 1.2 g.

Glycerin 6.0 g.

25 Methyl Hydroxybenzoate 0.2 g.

Purified Water B.P. to 100-00 ml. (B.P. = British Pharmacopeia)

The methyl hydroxybenzoate and glycerin are dissolved in 70 ml. of the water at 75°C. The sorbitan
30 monolaurate, polysorbate 20 and cetostearyl alcohol are melted together at 75°C and added to the aqueous

-45-

solution. The resulting emulsion is homogenized, allowed to cool with continuous stirring and the antibody or fragment thereof of the invention is added as a suspension in the remaining water. The whole

5 suspension is stirred until homogenized.

Eye Drop Composition

Antibody or fragment thereof of the invention
0.5 g.

Methyl Hydroxybenzoate 0.01 g.

10 Propyl Hydroxybenzoate 0.04 g.

Purified Water B.P. to 100-00 ml.

The methyl and propyl hydroxybenzoates are dissolved in 70 ml. purified water at 75°C and the resulting solution is allowed to cool. The antibody or
15 fragment thereof of the invention is then added, and the solution is sterilized by filtration through a membrane filter (0.022 μ m pore size), and packed aseptically into suitable sterile containers.

Composition for Administration by Inhalation

20 For an aerosol container with a capacity of 15-20 ml: mix 10 mg. of an antibody or fragment thereof of the invention with 0.2-0.5% of a lubricating agent, such as polysorbate 85 or oleic acid, and disperse such mixture in a propellant, such as freon, preferably in a
25 combination of (1,2 dichlorotetrafluoroethane) and difluorochloro-methane and put into an appropriate aerosol container adapted for either intranasal or oral inhalation administration.

Composition for Administration by Inhalation

30 For an aerosol container with a capacity of 15-20 ml: dissolve 10 mg. of an antibody or fragment thereof

-46-

of the invention in ethanol (6-8 ml.), add 0.1-0.2% of a lubricating agent, such as polysorbate 85 or oleic acid; and disperse such in a propellant, such as freon, preferably in combination of (1.2 dichlorotetra-

5 fluoroethane) and difluorochloromethane, and put into an appropriate aerosol container adapted for either intranasal or oral inhalation administration.

The antibodies and pharmaceutical compositions of the invention are particularly useful for parenteral
10 administration, i.e., subcutaneously, intramuscularly or intravenously. The compositions for parenteral administration will commonly comprise a solution of an antibody or fragment thereof of the invention or a cocktail thereof dissolved in an acceptable carrier,
15 preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., water, buffered water, 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by
20 conventional, well-known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the
25 antibody or fragment thereof of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight, and will be selected primarily based on fluid volumes, viscosities, etc.,
30 according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 Ml sterile buffered water, and 50 mg. of an antibody

-47-

or fragment thereof of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain 250 ml. of sterile Ringer's solution, and 150 mg. of an antibody or fragment thereof of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art, and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania, hereby incorporated by reference herein.

The antibodies (or fragments thereof) of the invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immune globulins and art-known lyophilization and reconstitution techniques can be employed.

Depending on the intended result, the pharmaceutical composition of the invention can be administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already suffering from a disease, in an amount sufficient to cure or at least partially arrest the disease and its complications. In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in a disease state to enhance the patient's resistance.

Single or multiple administrations of the pharmaceutical compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical composition of the invention should provide a quantity of the

-48-

altered antibodies (or fragments thereof) of the invention sufficient to effectively treat the patient.

It should also be noted that the antibodies of this invention may be used for the design and synthesis of

5 either peptide or non-peptide compounds (mimetics) which would be useful in the same therapy as the antibody. See, e.g., Saragovi et al., Science, 253, 792-795 (1991).

10 To further illustrate the invention, the following examples are provided. These examples are not intended, nor are they to be construed, as further limiting the invention.

Example 1

Recombinant immunoglobulin libraries displayed on
15 the surface of filamentous phage were first described by McCafferty et al, Nature, 348:552-554, 1990 and Barbas et al, Proc. Natl. Acad. Sci., USA 88:7978-7982, 1991. Using this technology, high affinity antibodies have been isolated from immune human recombinant libraries
20 (Barbas et al, Proc. Natl. Acad. Sci., USA 89:10164-10168, 1992). Although the phage display concept used is substantially similar to that described by Barbas, 1991, Id. the technique has been modified by the substitution of a unique vector for monkey libraries to
25 reduce the possibility of recombination and improve stability. This vector, pMS, Figure 1 contains a single lac promoter/operator for efficient transcription and translation of polycistronic heavy and light chain monkey DNA. This vector contains two different leader
30 sequences, the omp A (Movva et al, J. Biol. Chem., 255: 27-29, (1980), for the light chain and the pel B (Lei, J. Bact., 4379-109:4383 (1987) for the heavy chain Fd. Both leader sequences are translated into hydrophobic

-49-

signal peptides that direct the secretion of the heavy and light chain cloned products into the periplasmic space. In the oxidative environment of the periplasm, the two chains fold and disulfide bonds form to create stable Fab fragments. We derived the backbone of the vector from the phagemid bluescript. (Stratagene, La Jolla, CA). It contains the gene for the enzyme beta-lactamase that confers ampicillin (carbenicillin) resistance to bacteria that harbor pMS DNA. We also derived, from bluescript, the origin of replication of the multicopy plasmid ColE1 and the origin of replication of the filamentous bacteriophage f1. The origin of replication of phage f1 (the so-called intragenic region), signals the initiation of synthesis of single stranded pMS DNA, the initiation of capsid formation and the termination of RNA synthesis by viral enzymes. The replication and assembly of pMS DNA strands into phage particles requires viral proteins that must be provided by a helper phage. We have used helper phage VCSM13 which is particularly suited for this, since it also contains a gene coding for kanamycin resistance. Bacteria infected with VCSM13 and pMS can be selected by adding both kanamycin and carbenicillin to the growth medium. The bacteria will ultimately produce filamentous phage particles containing either pMS or VCSM13 genomes. Packaging of the helper phage is less efficient than that of pMS, resulting in a mixed phage population that contains predominately recombinant pMS phages. The ends of the phage pick up minor coat proteins specific to each end. Of particular interest here is the gene III product which is present in three to five copies at one end of the phage. The gene III product is 406 amino acid residues and is required for phage infection of *E. coli* via the F pili. The first

-50-

two domains of the heavy chain, the variable and the CH1 domain, are fused to the carboxy-terminal half of the gene III protein. This recombinant pili protein, directed by the pel B leader, is secreted to the

5 periplasm where it accumulates and forms disulfide bonds with the light chain before it is incorporated in the coat of the phage. Also, another vector contains a FLAG sequence engineered downstream of the gene III. The

10 FLAG is an 8 amino acid peptide expressed at the carboxy terminal of the Fd protein. We are using commercially available monoclonal anti-FLAG M2 for both purification and detection of phage Fab by ELISA (Brizzard, Bio

Techniques, 16(4):730-731, (1994)).

After constructing the vector pMS, we tested its

15 ability to produce phage bound Fab using control antibody genes. We cloned an anti-tetanus toxoid antibody, (obtained from Dr. Carlos Barbas), into pMS and transformed XLI-blue. We co-infected our cells with VCSM13 and generated phage displaying the anti-tetanus

20 toxoid antibody. We performed efficiency experiments where anti-tetanus toxoid phage were combined with phage beading an irrelevant antibody at 1:100,000. We performed three rounds of panning by applying 50 μ l of the mixed phage to antigen (tetanus toxoid) coated

25 polystyrene wells. Non-adherent phage were washed off and the adherent phage were eluted with acid. The eluted phage were used to infect a fresh aliquot of XLI-Blue bacteria and helper phage was added. After overnight amplification, phage were prepared and again

30 panned on antigen coated plates. After three rounds of panning, we were able to show that we had successfully enriched for the anti-tetanus toxoid phage. The success of this technology also depends on the ability to prepare soluble Fabs for characterization of the final

-51-

panned product. This was achieved by excising gene III from the pMS DNA using the restriction enzyme Nhe I followed by re-ligation. After the gene III was excised, the Fab was no longer displayed on the phage surface but accumulated in the periplasmic space.

Lysates were prepared from bacteria expressing soluble Fab and tested for antigen specificity using an ELISA. High levels of soluble Fab were detected.

In order to adapt phage display technology for use with macaque libraries, we developed specific primers for PCR amplifying monkey immunoglobulin genes. These were based on macaque sequences we obtained while developing the PRIMATIZED® antibody technology (See, 08/379,072, incorporated by reference herein) and databases containing human sequences. (Kabat et al, (1991), "Sequences of Proteins of Immunological Interest," U.S. Dept. of Health and Human Services, National Institute of Health).

We developed three sets of primers to cover amplification of the macaque repertoire. Our first set of primers was designed for amplification of the heavy chain VH and CH1 (Fd) domains. It consisted of a 3' CH1 domain primer and six 5' VH family specific primers that bind in the framework 1 region. Our second set of primers, for amplifying the whole lambda chain, covers the many lambda chain subgroups. It consists of a 3' primer and three 5' degenerate primers that bind in the VL framework 1 region. Our third set of primers was designed for amplification of the kappa chain subgroups. It consists of one 3' primer and five VK framework 1 primers. Using each of these sets, PCR parameters were optimized to obtain strong enough signals from each primer pair so that ample material was available for cloning of the library. We recently created macaque

-52-

combinatorial libraries in our pMS vector using these optimized PCR conditions. Bone marrow biopsies were taken from CD4 immune monkeys as the source of immunoglobulin RNA. The libraries contained
5 approximately 10^6 members and are currently being panned or specific binders on antigen coated wells.

Example 2

Development of B7/CTLA-4 Reagents

We have generated a number of reagents for the
10 purpose of immunizing monkeys, developing binding and functional assays *in vitro*, screening heterohybridomas and panning phage libraries. Table 1 lists each reagent and its intended purpose. In the case of B7.1, RNA was extracted from SB cells and converted to cDNA using
15 reverse transcriptase. The first strand cDNA was PCR amplified using B7.1 specific primers and cloned into IDEC's NEOSPLA mammalian expression vectors. CHO cells were transfected with B7.1 NEOSPLA DNA and clones expressing membrane associated B7.1 were identified.
20 The B7.1 fusion protein was generated similarly, except that the PCR amplified B7.1 gene was cloned into a NEOSPLA cassette vector containing the human CH2 and CH3 immunoglobulin genes. CHO cells were transformed with the B7.1/Ig NEOSPLA DNA and stable clones secreting
25 B7.1/Ig fusion protein were amplified. In general, the B7.2 and CTLA4 reagents were generated in the same manner, except that for B7.2 the RNA was isolated from human spleen cells that had been stimulated 24 hours with anti-Ig and IL-4, and for the CTLA4 constructs the
30 gene source was PHA activated human T cells.

-53-

Table 1

| Reagent | Purpose | CHO Expression |
|------------------------|-----------------------------|-----------------|
| Soluble B7.1 | Immunization, immunoassays | Yes |
| B7.1 Transfectant | Screening, ELISA | Yes |
| B7.1/Ig Fusion Protein | Inhibition studies, panning | Yes |
| B7.2 Transfectant | Screening, ELISA | Yes |
| B7.2/Ig Fusion Protein | Inhibition studies, panning | To be completed |
| CTLA4 Transfectant | Inhibition studies | To be completed |
| CTLA4/Ig | Inhibition studies | To be completed |

5 The availability of these reagents, together with
10 monoclonal antibodies to B7.1 (L3074) (Becton Dickinson,
1994) and B7.2 (Fun-1 (Engel et al, Blood, 84, 1402-
1407, (1994) and purified goat and rabbit antisera,
specifically developed to detect monkey Fab fragments,
15 facilitates identification of antibodies having the
desired properties.

Example 3

Generation of a Phage Display Library

Recombinant phage display libraries are generated
20 from B7.1 and B7.2 immune monkeys. Lymph node and bone
marrow biopsies are performed 7-12 days after
immunization to harvest RNA rich B cells and plasma
cells. RNA is isolated from the lymphocytes using the
method described by Chomczynski Anal. Biochem., 162(1),
25 156-159, (1987). RNA is converted to cDNA using an
oligo dT primer and reverse transcriptase. The first
strand cDNA is divided into aliquots and PCR amplified
using the sets of kappa, lambda, and heavy chain Fd
region primers described earlier and either Pfu
30 polymerase (Stratagene, San Diego) or Taq polymerase
(Promega, Madison). The heavy chain PCR amplified
products are pooled, cut with Xho VSpe I restriction
enzymes and cloned into the vector pMS. Subsequently,

-54-

the light chain PCR products are pooled, cut with Sac I/Xba I restriction enzymes, and cloned to create the recombinant library. XLI-Blue *E. coli* is transformed with the library DNA and super-infected with VCSM13 to produce the phage displaying antibodies. The library is panned four rounds on polystyrene wells coated with B7.1 or B7.2 antigen. Individual phage clones from each round of panning are analyzed. The pMS vector DNA is isolated and the gene III excised.

10 Soluble Fab fragments are generated and tested in ELISA for binding to B7.1 and B7.2.

Example 4

Characterization of Phage Fab Fragments

The monkey phage Fab fragments are characterized for their specificity and the ability to block B7.1-Ig and B7.2-Ig binding to CTLA-4-Ig or CTLA-4 transfected cells. Phage fragments are also characterized for cross-reactivity after first panning for 4 rounds on the B7 species used for immunization in order to select for high affinity fragments. Fab fragments identified from four rounds of panning either on B7.1 or B7.2 antigen coated surfaces are scaled up by infection and grown in 24 hour fermentation cultures of *E. coli*. Fragments are purified by Kodak FLAG binding to a anti-FLAG affinity column. Purified phage Fabs are tested for affinity by an ELISA based direct binding modified Scatchard analysis (Kato et al, J. Chem. BioEng., 76:451-454, (1993)) using Goat anti-monkey Fab antibodies or anti-FLAG MAb conjugated with horseradish peroxidase. The anti-monkey Fab reagents will be absorbed against human heavy chain constant region Ig to remove any cross-reactivity to B7-Ig. Kd values are calculated for each

-55-

fragment after measurements of direct binding to B7.1-Ig or B7.2-Ig coated plates.

Example 5

Phage Fab Fragment Blocking of CTLA-4/B7 Binding

5 Fab fragments most effectively blocking the binding of B7-Ig at the lowest concentrations are selected as lead candidates. Selections are made by competing off ^{125}I -B7-Ig binding to CTLA-4-Ig or CTLA-4 transfected
10 cells. Additional selection criteria include, blocking of mixed lymphocyte reaction (MLR), as measured by inhibiting 3H-thymidine uptake in responder cells (Azuma et al, J. Exp. Med., 177:845-850,; Azuma et al, Nature, 301:76-79, (1993)) and direct analysis of IL-2
15 production using IL-2 assay kits. The three or four candidates which are most effective in inhibiting of MLR and CTLA-4 binding assays are chosen for cloning into the above-described mammalian expression vector for transfection into CHO cells and expression of chimeric monkey/human antibodies.

Example 6

Generation of Monkey Heterohybridomas

20 Monkey heterohybridomas secreting monoclonal antibodies are generated from existing immunized animals whose sera tested positive for B7.1 and/or B7.2. Lymph
25 node biopsies are taken from animals positive to either, or both, antigens. The method of hybridoma production is similar to the established method used for the generation of monkey anti-CD4 antibodies (Newman, 1992(Id.)). Monkeys with high serum titers will have
30 sections of inguinal lymph nodes removed under

-56-

anesthesia. Lymphocytes are washed from the tissue and fused with KH6/B5 heteromyeloma cells (Carrol et al, J. Immunol. Meth., 89:61-72, (1986)) using polyethylene glycol (PEG). Hybridomas are selected on H.A.T. media
5 and stabilized by repeated subcloning in 96 well plates.

Monkey monoclonal antibodies specific for B7.1 antigen are screened for cross-reactivity to B7.2. Monkey anti-B7 antibodies will be characterized for blocking of B7/CTLA-4 binding using the ^{125}I -B7-Ig
10 binding assay. Inhibition of MLR by ^3H -Thymidine uptake and direct measurement of IL-2 production is used to select three candidates. Two candidates will be brought forward in Phase II studies and expressed in CHO cells while repeating all functional studies. For the
15 purposes of developing an animal model for *in vivo* pharmacology, anti-B7 antibodies will be tested on cells of several animal species. The establishment of an animal model will allow preclinical studies to be carried out for the selected clinical indication.

20 Example 7

As discussed *supra*, using the above heterohybridoma methods, 4 lead monkey anti-B7.1 antibodies have been identified: 16C10, 7B6, 7C10 and 20C9. These antibodies were characterized as follows:

25 Scatchard analysis showed that the apparent affinity constants (K_d) for the monkey antibodies binding to B7-Ig coated plates were approximated to be:

30 a: 7C10: $6.2 \times 10^{-9}\text{M}$
b: 16C10: $8.1 \times 10^{-9}\text{M}$
c: 7B6: $10.7 \times 10^{-9}\text{M}$
d: 20C9: $16.8 \times 10^{-9}\text{M}$

The antibodies were tested *in vitro* in a mixed lymphocyte reaction assay (MLR). The MLR showed

-57-

that all 4 anti-B7.1 antibodies inhibit IL-2
production to different extents:

| | | | |
|---|----|--------|----------------|
| | a: | 7B6: | 5.0 μ g/Ml |
| | b: | 16C10: | 0.1 μ g/Ml |
| 5 | c: | 20C9: | 2.0 μ g/Ml |
| | d: | 7C10: | 5.0 μ g/Ml |

The monkey anti-B7.1 antibodies were tested for
their ability to bind B7 on human peripheral blood
lymphocytes (PBL). FACS analysis showed that all 4
10 monkey antibodies tested positive.

Monkey antibodies 16C10, 7B6, 7C10 and 20C9 were
tested for Clq binding by FACS analysis. Results
showed 7C10 monkey Ig had strong human Clq binding
after incubating with B7.1 CHO-transfected cells.
15 16C10 was also positive, while 20C9 and 7B6 monkey
antibodies were negative.

Example 8

Using the primatized antibody methodology
incorporated by reference to commonly assigned U.S.
20 Serial No. 08/379,072, and using the NEOSPLA vector
system shown in Figure 2, the heavy and light variable
domains of 7C10, 7B6 and 16C10 were cloned and
primatized forms thereof have been synthesized in CHO
cells using the NEOSPLA vector system. The amino acid
25 and nucleic acid sequences for the primatized 7C10 light
and heavy chain, 7B6 light and heavy chain, and 16C10
light and heavy chain are respectively shown in Figures
3a, 3b, 4a, 4b, 5a and 5b.

-58-

Example 9

Confirming experiments on the non-cross-reactivity of the CTLA-4 and PRIMATIZED® antibody binding sites on B7.1.

5 ~~In competitive binding assays using biotinylated~~
CTLA-4Ig (Figure 6), unlabeled primatized 16C10 (i.e., P16C10) was unable to block CTLA-4Ig binding to B7.1 transfected CHO cells. It can be seen that unlabeled CTLA-4Ig and unlabeled B7.1 effectively compete under
10 these conditions.

In a second experiment using Biotinylated P16C10, the same conclusions can be made. In the experiment shown in Figure 7, binding of P16C10-Biotin is inhibited by both unlabeled P16C10 and B7.1Ig, but not by CTLA-
15 4Ig. Although CTLA-4Ig is reported to be as much as 4-10 fold higher in affinity ($K_d=0.4$ nM; Morton et al., J. Immunol. 156:1047-1054 (1996)), there is no significant inhibition of P16C10 binding even at CTLA-4Ig concentrations as high as 100 fold excess.

20 Similar results were obtained using the primatized antibody 7C10 (P7C10) when it was substituted for P16C10 in the experiments (data not provided).

Example 10

25 Comparing the ability of L307.4 and BB-1 mouse antibodies to bind to B7 CHO cells in the presence of CTLA-4Ig.

The binding of L307.4 and BB-1 murine anti-B7
antibody in the presence of CTLA-4Ig was studied in order to determine whether the mouse antibody binding
30 sites overlapped with the CTLA-4 binding site. Competition assay experiments using P16C10-Biotin, L307.4-Biotin and CTLA-4Ig-Biotin were done to insure that affinity differences did not prevent detection of

-59-

competitive binding. The results are shown in Figures 8 and 9.

The results of Figure 8 confirm earlier studies that the mouse antibody BB-1 does not compete with P16C10. These results also show that there is some cross-reactivity to L307.4 of approximately 50%. The results of Figure 8 confirm that BB-1 and L307.4 both compete with each other and that BB-1 completely blocks binding of CTLA-4Ig-Biotin to B7.1 transfected CHO cells. BB-1 does not significantly affect P16C10 binding to B7.1 positive CHO cells.

The results shown in Figure 9 indicate better than 50% competition when CTLA-4Ig-Biotin is used in the binding experiment. Figure 9 shows that CTLA-4Ig-Biotin is effectively blocked by all B7.1 inhibitors except P16C10, therefore P16C10 recognizes a unique binding determinate on B7.1 which allows the normal CTLA-4 ligand binding in the generation of negative signals. Earlier functional studies (data not shown) suggest a weakened ability of L307.4 to block IL-2 production in allogeneic MLR, which correlates with the hypothesis that it may interfere with CTLA-4 negative signaling. It is not clear how many of the other murine antibodies reported in the literature give complete inhibition of CTLA-4 binding; however, this issue may be important in defining the true functional mechanisms of B7.1 and B7.2 specific antibodies.

These results confirm earlier studies using B7-Ig in competition with P16C10-Biotin for binding to B7.1 transfected CHO cells. The studies also confirm earlier observations of no inhibition of the P16C10 by CTLA-4Ig. These results are highly suggestive that the primate antibodies are specific for a unique B7.1 epitope independent of the CTLA-4 binding site which interacts

-60-

primarily with CD28. This type of interaction would provide a benefit, since it has the ability to block binding of B7.1 to CD28 receptors while still allowing the negative signaling function of CTLA-4 to occur uninhibited. This perceived interaction may lead to a down regulation of the overall T cell activation response regardless of the predominance of either Th1 or Th2 phenotypes.

Similar results were obtained using P7C10 when it was substituted for P16C10 in the experiments (data not provided).

Example 11

Experiment demonstrating the ability of P16C10 to bind and block B7.1 interactions with CD28 receptor.

An experiment to determine if P16C10 binding of B7.1 can block the interaction of B7.1 with CD28 was attempted by radiolabeling B7.1Ig with ^{125}I , followed by competitive binding to CD28 positive non-activated peripheral blood T lymphocytes. The results shown in Figure 10 demonstrate that the radiolabeled B7.1Ig binds specifically to the T cells, as confirmed by inhibition with unlabeled B7.1Ig. The results also show that CTLA-4Ig, anti-CD28 and P16C10 are all capable of blocking this interaction. The results further confirm that P16C10 blocks binding of the CD28/B7 interaction with an IC_{50} of $< 1 \text{ ug/mL}$.

The above results were obtained under conditions where no membrane associated CTLA-4 was expressed (Linsley et al., J. Exp. Med. 173:721-730 (1991)) and confirmed by blocking with the anti-CD28 antibody.

Similar results were obtained using P7C10 when it was substituted for P16C10 in the experiments (data not provided).

-61-

It is expected that these primatized antibodies, given their probable low antigenicity and human effector function, will be well suited as therapeutics. In fact, it has recently been shown that primatized 16C10

5 exhibits human Clq binding.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments of the invention described herein. Such equivalents are
10 intended to be embraced by the following claims.

-62-

What Is Claimed Is:

1. A monoclonal antibody which specifically binds to B7.1 antigen (CD80) or B7.2 antigen (CD86), and which antibody inhibits the binding of said B7.1 or B7.2 antigen to CD28.

2. The monoclonal antibody of claim 1 wherein said antibody specifically binds to B7.1 antigen (CD80).

3. The monoclonal antibody of claim 2 wherein said antibody does not inhibit the binding of B7.1 antigen to CTLA-4.

4. The monoclonal antibody of claim 1 wherein said antibody specifically binds to B7.2 antigen (CD86).

5. The monoclonal antibody of claim 4 wherein said antibody does not inhibit the binding of B7.2 antigen to CTLA-4.

6. The monoclonal antibody of claim 1 which inhibits the production of IL-2 by T cells.

7. The monoclonal antibody of claim 2 which selectively inhibits the interaction of B and T cells via the CD28/B7.1 pathway.

8. The monoclonal antibody of claim 4 which selectively inhibits the interaction of B and T cells via the CD28/B7.2 pathway.

9. The monoclonal antibody of claim 1 which is capable of inhibiting in vitro the production of IL-2 by T lymphocytes.

-63-

10. The monoclonal antibody of claim 9 wherein said antibody is capable of inhibiting IL-2 production when added to a T lymphocyte containing culture at a concentration of at least 10 $\mu\text{g/ml}$.

11. A monoclonal antibody which binds to the same epitope on B7.1 as 16C10 or 7C10, or which monoclonal antibody inhibits the interaction of 16C10 or 7C10 with B7.1.

12. The monoclonal antibody of claim 1 which is a primatized antibody.

13. The monoclonal antibody of claim 1 which is a human, chimeric mouse/human, or humanized antibody.

14. The monoclonal antibody of claim 1 wherein said B7.1 is human B7.1.

15. The monoclonal antibody of claim 1 wherein said B7.2 is human B7.2.

16. A method of treating a disease involving T cell/B cell interactions comprising administering an amount of a monoclonal antibody according to claim 2 sufficient to inhibit the binding of B cells and T cells via the B7.1/CD28 pathway.

17. A method of treating a disease involving T cell/B cell interactions comprising administering an amount of a monoclonal antibody according to claim 4 sufficient to inhibit the binding of B cells and T cells via the B7.2/CD28 pathway.

-64-

18. The method of claim 16 wherein said disease is an autoimmune disorder.

19. The method of claim 17 wherein said disease is an autoimmune disorder.

20. The method of claim 16 wherein said disease is selected from the group consisting of idiopathic thrombocytopenia purpura, systemic lupus erythematosus, type 1 diabetes mellitus, rheumatoid arthritis, psoriasis, aplastic anemia, inflammatory bile disease, allergy and multiple sclerosis.

21. The method of claim 17 wherein said disease is selected from the group consisting of idiopathic thrombocytopenia purpura, systemic lupus erythematosus, type 1 diabetes mellitus, rheumatoid arthritis, psoriasis, aplastic anemia, inflammatory bile disease, allergy and multiple sclerosis.

22. The method of claim 16 wherein said disease is graft-versus-host disease.

23. The method of claim 17 wherein said disease is graft-versus-host disease.

24. The method of claim 16 wherein said disease is selected from the group consisting of B cell lymphoma, infectious diseases, and inflammatory diseases.

25. The method of claim 17 wherein said disease is selected from the group consisting of B cell lymphoma, infectious diseases, and inflammatory diseases.

-65-

26. A pharmaceutical composition suitable for treatment of a disease treatable by inhibition of B7:CD28 binding which comprises an antibody according to claim 1.

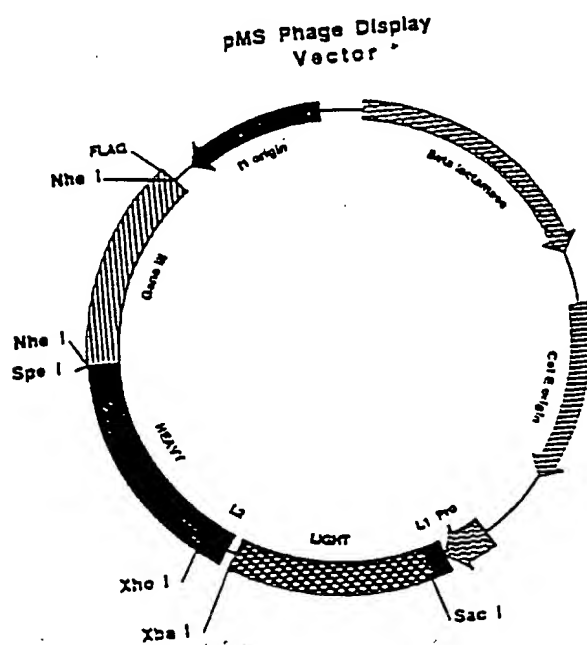
27. The method of claim 16 wherein the antibody is administered in combination with other recombinant protein or small molecule immunosuppressants.

28. The method of claim 17 wherein the antibody is administered in combination with other recombinant protein or small molecule immunosuppressants.

1/16

FIGURE 1

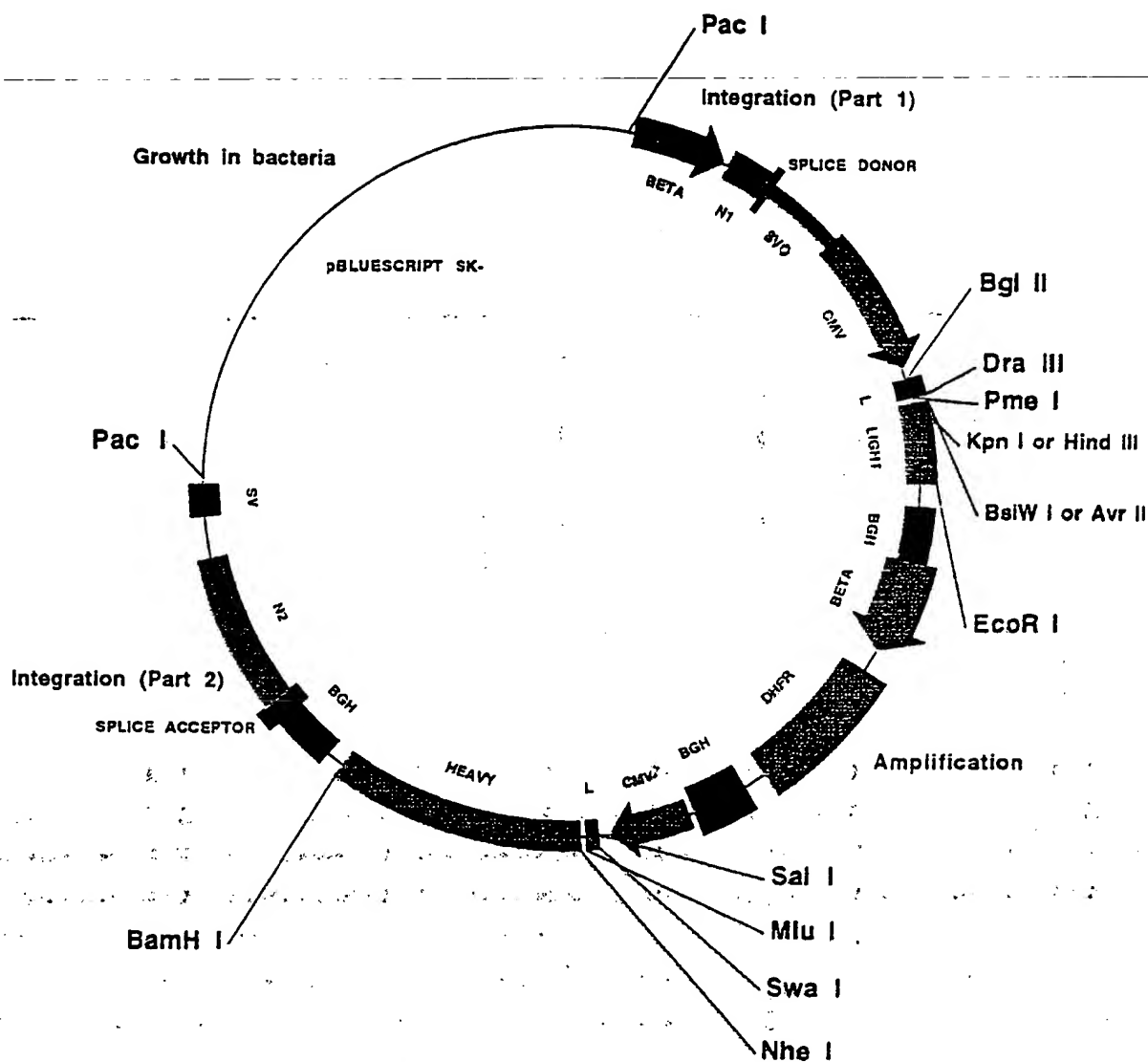
Figure 1: pMS Phage Display Vector. Pro = Lac Z promoter. L1 = Omp A leader. L2 = Pel B leader. Light = light chain. Heavy = heavy chain.



2/16

FIGURE 2

NEOSPLA



- CMV = Cytomegalovirus promoter/enhancer
 BETA = Mouse Beta globin major promoter
 SVO = SV40 origin
 BGH = Bovine growth hormone polyadenylation
 SV = SV40 polyadenylation
 N1 = Neomycin phosphotransferase exon 1
 N2 = Neomycin phosphotransferase exon 2
 LIGHT = Human immunoglobulin kappa or lambda constant region
 DHFR = Dihydrofolate Reductase
 HEAVY = Human immunoglobulin gamma 1 or gamma 4 PE constant region
 L = Leader

[illegible]

4/16

FIGURE 3b

Length of 7C10 heavy/Primatized: 1431 bp; Listed from: 1 to: 1431;
 Translated from: 1 to: 1429 (Entire region);
 Genetic Code used: Universal; Fri, May 26, 1995 11:11 AM

```

Frame 1  M  K  H  L  W  F  F  L  L  L  V  A  A  P  R  W  V  L  S
          ATG AAA CAC CTG TGG TTC CTC CTC CTG GTG GCA GCT CCC AGA TGG GTC CTG TCC
                9                18                27                36                45                54

Q  V  K  L  Q  Q  W  G  E  G  L  L  Q  P  S  E  T  L  S  R  T
CAG GTG AAG CTG CAG CAG TGG GGC GAA GGA CTT CTG CAG CCT TCG GAG ACC CTG TCC CGC ACC
    63          72          81          90          99          108          117

C  V  V  S  G  G  S  I  S  G  Y  Y  Y  W  T  W  I  R  Q  T  P
TGC GTT GTC TCT GGT GGC TCC ATC AGC GGT TAC TAC TAC TGG ACC TGG ATC CGC CAG ACC CCA
    126        135        144        153        162        171        180

G  R  G  L  E  W  I  G  H  I  Y  G  N  G  A  T  T  N  Y  N  P
GGG AGG GGA CTG GAG TGG ATT GGC CAT ATT TAT GGT AAT GGT GCG ACC ACC AAC TAC AAT CCC
    189        198        207        216        225        234        243

S  L  K  S  R  V  T  I  S  K  D  T  S  K  N  Q  F  F  L  N  L
TCC CTC AAG AGT CGA GTC ACC ATT TCA AAA GAC ACG TCC AAG AAC CAG TTC TTC CTG AAC TTG
    252        261        270        279        288        297        306

N  S  V  T  D  A  D  T  A  V  Y  Y  C  A  R  G  P  R  P  D  C
AAT TCT GTG ACC GAC GCG GAC ACG GCC GTC TAT TAC TGT GCG AGA GGC CCT CGC CCT GAT TGC
    315        324        333        342        351        360        369

T  T  I  C  Y  G  G  W  V  D  V  W  G  P  G  D  L  V  T  V  S
ACA ACC ATT TGT TAT GGC GGC TGG GTC GAT GTC TGG GGC CCG GGA GAC CTG GTC ACC GTC TCC
    378        387        396        405        414        423        432

S  A  S  T  K  G  P  S  V  F  P  L  A  P  S  S  K  S  T  S  G
TCA GCT AGC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG
    441        450        459        468        477        486        495

G  T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W
GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG
    504        513        522        531        540        549        558

N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L
AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC
    567        576        585        594        603        612        621

Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y  I  C
TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG ACC TAC ATC TGC
    630        639        648        657        666        675        684

N  V  N  H  K  P  S  N  T  K  V  D  K  K  A  E  P  K  S  C  D
AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GCA GAG CCC AAA TCT TGT GAC
    693        702        711        720        729        738        747

K  T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L
AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC
    756        765        774        783        792        801        810

P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V
TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG
    819        828        837        846        855        864        873

D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V
ATG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG
    882        891        900        909        918
  
```

5/16

FIGURE 3b (Continued)

| | | | | | | | | | | | | | | | | | | | | |
|------|-----|-----|-----|------|-----|-----|------|-----|-----|------|-----|-----|------|-----|-----|------|-----|-----|------|-----|
| H | N | A | K | T | K | P | R | E | E | Q | Y | N | S | T | Y | R | V | V | S | V |
| CAT | AAT | GCC | AAG | ACA | AAG | CCG | CGG | GAG | GAG | CAG | TAC | AAC | AGC | ACG | TAC | CGT | GTG | GTC | AGC | GTC |
| 945 | | | | 954 | | | 963 | | | 972 | | | 981 | | | 990 | | | 999 | |
| L | T | V | L | H | Q | D | W | L | N | G | K | E | Y | K | C | K | V | S | N | K |
| CTC | ACC | GTC | CTG | CAC | CAG | GAC | TGG | CTG | AAT | GGC | AAG | GAG | TAC | AAG | TGC | AAG | GTC | TCC | AAC | AAA |
| 1008 | | | | 1017 | | | 1026 | | | 1035 | | | 1044 | | | 1053 | | | 1062 | |
| A | L | P | A | P | I | E | K | T | I | S | K | A | K | G | Q | P | R | E | P | Q |
| GCC | CTC | CCA | GCC | CCC | ATC | GAG | AAA | ACC | ATC | TCC | AAA | GCC | AAA | GGG | CAG | CCC | CGA | GAA | CCA | CAG |
| 1071 | | | | 1080 | | | 1089 | | | 1098 | | | 1107 | | | 1116 | | | 1125 | |
| V | Y | T | L | P | P | S | R | D | E | L | T | K | N | Q | V | S | L | T | C | L |
| GTG | TAC | ACC | CTG | CCC | CCA | TCC | CGG | GAT | GAG | CTG | ACC | AAG | AAC | CAG | GTC | AGC | CTG | ACC | TGC | CTG |
| 1134 | | | | 1143 | | | 1152 | | | 1161 | | | 1170 | | | 1179 | | | 1188 | |
| V | K | G | F | Y | P | S | D | I | A | V | E | W | E | S | N | G | Q | P | E | N |
| GTC | AAA | GGC | TTC | TAT | CCC | AGC | GAC | ATC | GCC | GTG | GAG | TGG | GAG | AGC | AAT | GGG | CAG | CCG | GAG | AAC |
| 1197 | | | | 1206 | | | 1215 | | | 1224 | | | 1233 | | | 1242 | | | 1251 | |
| N | Y | K | T | T | P | P | V | L | D | S | D | G | S | F | F | L | Y | S | K | L |
| AAC | TAC | AAG | ACC | ACG | CCT | CCC | GTG | CTG | GAC | TCC | GAC | GGC | TCC | TTC | TTC | CTC | TAC | AGC | AAG | CTC |
| 1260 | | | | 1269 | | | 1278 | | | 1287 | | | 1296 | | | 1305 | | | 1314 | |
| T | V | D | K | S | R | W | Q | Q | G | N | V | F | S | C | S | V | M | H | E | A |
| ACC | GTG | GAC | AAG | AGC | AGG | TGG | CAG | CAG | GGG | AAC | GTC | TTC | TCA | TGC | TCC | GTG | ATG | CAT | GAG | GCT |
| 1323 | | | | 1332 | | | 1341 | | | 1350 | | | 1359 | | | 1368 | | | 1377 | |
| L | H | N | H | Y | T | Q | K | S | L | S | L | S | P | G | K | . | | | | |
| CTG | CAC | AAC | CAC | TAC | ACG | CAG | AAG | AGC | CTC | TCC | CTG | TCT | CCG | GGT | AAA | TGA | | | | |
| 1386 | | | | 1395 | | | 1404 | | | 1413 | | | 1422 | | | 1431 | | | | |

FIGURE 4a

Length of 7B6 light/Primatized: 720 bp; Listed from: 1 to: 720;

Translated from: 1 to: 718 (Entire region);

Genetic Code used: Universal; Fri, May 26, 1995 11:10 AM

| | | | | | | | | | | | | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Frame 1 | M | S | L | P | A | Q | L | L | G | L | L | C | V | P | G | S | S | | | |
| | ATG | AGC | CTC | CCT | GCT | CAG | CTC | CTC | GGG | CTG | CTA | TTG | CTC | TGC | GTC | CCC | GGG | TCC | AGT | |
| | | | 9 | | | 18 | | | 27 | | | 36 | | | 45 | | | 54 | | |
| G | E | V | V | M | T | Q | S | P | L | S | L | P | I | T | P | G | E | P | A | S |
| GGG | GAA | GTT | GTG | ATG | ACT | CAG | TCT | CCA | CTG | TCC | CTT | CCC | ATC | ACA | CCT | GGA | GAG | CCG | GCC | TCC |
| | 63 | | | 72 | | | 81 | | | 90 | | | 99 | | | 108 | | | 117 | |
| I | S | C | R | S | S | Q | S | L | K | H | S | N | G | D | T | F | L | S | W | Y |
| ATC | TCC | TGT | AGG | TCT | AGT | CAA | AGC | CTT | AAA | CAC | AGT | AAT | GGA | GAC | ACC | TTC | CTG | AGT | TGG | TAT |
| | 126 | | | 135 | | | 144 | | | 153 | | | 162 | | | 171 | | | 180 | |
| Q | Q | K | P | G | Q | P | P | R | L | L | I | Y | K | V | S | N | R | D | S | G |
| CAG | CAG | AAG | CCA | GGC | CAA | CCT | CCA | AGG | CTC | CTG | ATT | TAT | AAG | GTT | TCT | AAC | CGG | GAC | TCT | GGG |
| | 189 | | | 198 | | | 207 | | | 216 | | | 225 | | | 234 | | | 243 | |
| V | P | D | R | F | S | G | S | G | A | G | T | D | F | T | L | K | I | S | A | V |
| GTC | CCA | GAC | AGA | TTC | AGC | GGC | AGT | GGG | GCA | GGG | ACA | GAT | TTC | ACA | CTG | AAA | ATC | AGC | GCA | GTG |
| | 252 | | | 261 | | | 270 | | | 279 | | | 288 | | | 297 | | | 306 | |
| E | A | E | D | V | G | V | Y | F | C | G | Q | G | T | R | T | P | P | T | F | G |
| GAG | GCT | GAA | GAT | GTT | GGG | GTT | TAT | TTC | TGC | GGG | CAA | GGT | ACA | AGG | ACT | CCT | CCC | ACT | TTC | GGC |
| | 315 | | | 324 | | | 333 | | | 342 | | | 351 | | | 360 | | | 369 | |
| G | G | T | K | V | E | I | K | R | T | V | A | A | P | S | V | F | I | F | P | P |
| GGA | GGG | ACC | AAG | GTG | GAA | ATC | AAA | CGT | ACG | GTG | GCT | GCA | CCA | TCT | GTC | TTC | ATC | TTC | CCG | CCA |
| | 378 | | | 387 | | | 396 | | | 405 | | | 414 | | | 423 | | | 432 | |
| S | D | E | Q | L | K | S | G | T | A | S | V | V | C | L | L | N | N | F | Y | P |
| TCT | GAT | GAG | CAG | TTG | AAA | TCT | GGA | ACT | GCC | TCT | GTT | GTG | TGC | CTG | CTG | AAT | AAC | TTC | TAT | CCC |
| | 441 | | | 450 | | | 459 | | | 468 | | | 477 | | | 486 | | | 495 | |
| R | E | A | K | V | Q | W | K | V | D | N | A | L | Q | S | G | N | S | Q | E | S |
| AGA | GAG | GCC | AAA | GTA | CAG | TGG | AAG | GTG | GAT | AAC | GCC | CTC | CAA | TGC | GGT | AAC | TCC | CAG | GAG | AGT |
| | 504 | | | 513 | | | 522 | | | 531 | | | 540 | | | 549 | | | 558 | |
| V | T | E | Q | D | S | K | D | S | T | Y | S | L | S | S | T | L | T | L | S | K |
| GTC | ACA | GAG | CAG | GAC | AGC | AAG | GAC | AGC | ACC | TAC | AGC | CTC | AGC | AGC | ACC | CTG | ACG | CTG | AGC | AAA |
| | 567 | | | 576 | | | 585 | | | 594 | | | 603 | | | 612 | | | 621 | |
| A | D | Y | E | K | H | K | V | Y | A | C | E | V | T | H | Q | G | L | S | S | P |
| GCA | GAC | TAC | GAG | AAA | CAC | AAA | GTC | TAC | GCC | TGC | GAA | GTC | ACC | CAT | CAG | GGC | CTG | AGC | TGC | CCC |
| | 630 | | | 639 | | | 648 | | | 657 | | | 666 | | | 675 | | | 684 | |
| V | T | K | S | F | N | R | G | E | C | | | | | | | | | | | |
| GTC | ACA | AAG | AGC | TTC | AAC | AGG | GGA | GAG | TGT | TGA | | | | | | | | | | |
| | 693 | | | 702 | | | 711 | | | 720 | | | | | | | | | | |

7/16

FIGURE 4b

Length of 7B6 heavy/Primatized: 1437 bp; Listed from: 1 to: 1437;
Translated from: 1 to: 1435 (Entire region);
Genetic Code used: Universal; Fri, May 26, 1995 11:09 AM

| | | | | | | | | | | | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Frame 1 | M | G | W | S | L | I | L | L | F | L | V | A | V | A | T | R | V | Q | C |
| | ATG | GGT | TGG | AGC | CTC | ATC | TTG | CTC | TTC | CTT | GTC | GCT | GTT | GCT | ACG | CGT | GTC | CAG | TGT |
| | | | 9 | | | 18 | | | 27 | | | 36 | | | 45 | | | 54 | |
| E | V | Q | L | V | E | S | G | G | L | V | Q | P | G | G | S | L | R | V | S |
| SAG | GTG | CAA | CTG | GTG | GAG | TCT | GGG | GGA | GGC | TTG | GTC | CAG | CCT | GGC | GGG | TCC | CTG | AGA | GTC |
| | 63 | | 72 | | | 81 | | | 90 | | | 99 | | | 108 | | | 117 | |
| C | A | V | S | G | F | T | F | S | D | H | Y | M | Y | W | F | R | Q | A | P |
| IGT | GCA | GTC | TCT | GGA | TTC | ACC | TTC | AGT | GAC | CAC | TAC | ATG | TAT | TGG | TTC | CGC | CAG | GCT | CCA |
| | 126 | | 135 | | | 144 | | | 153 | | | 162 | | | 171 | | | 180 | |
| K | G | P | E | W | V | G | F | I | R | N | K | P | N | G | G | T | T | E | Y |
| AAG | GGG | CCG | GAA | TGG | GTA | GGT | TTC | ATT | AGA | AAC | AAA | CCG | AAC | GGT | GGG | ACA | ACA | GAA | TAC |
| | 189 | | 198 | | | 207 | | | 216 | | | 225 | | | 234 | | | 243 | |
| A | S | V | K | D | R | F | T | I | S | R | D | D | S | K | S | I | A | Y | L |
| CGC | TCT | GTG | AAA | GAC | AGA | TTC | ACC | ATC | TCC | AGA | GAT | GAT | TCC | AAA | AGC | ATC | GCC | TAT | CTG |
| | 252 | | 261 | | | 270 | | | 279 | | | 288 | | | 297 | | | 306 | |
| M | S | S | L | K | I | E | D | T | A | V | Y | Y | C | T | T | S | Y | I | S |
| ATG | AGC | AGC | CTG | AAA | ATC | GAG | GAC | ACG | GCC | GTC | TAT | TAC | TGT | ACT | ACA | TCC | TAC | ATT | TCA |
| | 315 | | 324 | | | 333 | | | 342 | | | 351 | | | 360 | | | 369 | |
| C | R | G | G | V | C | Y | G | G | Y | F | E | F | W | G | Q | G | A | L | V |
| IGT | CGG | GGT | GGT | GTC | TGC | TAT | GGA | GGT | TAC | TTC | GAA | TTC | TGG | GGC | CAG | GGC | GCC | CTG | GTC |
| | 378 | | 387 | | | 396 | | | 405 | | | 414 | | | 423 | | | 432 | |
| V | S | S | A | S | T | K | G | P | S | V | F | P | L | A | P | S | S | K | S |
| GTC | TCC | TCA | GCT | AGC | ACC | AAG | GGC | CCA | TGC | GTC | TTC | CCC | CTG | GCA | CCC | TCC | TCC | AAG | AGC |
| | 441 | | 450 | | | 459 | | | 468 | | | 477 | | | 486 | | | 495 | |
| S | G | G | T | A | A | L | G | C | L | V | K | D | Y | F | P | E | P | V | T |
| TCT | GGG | GGC | ACA | GCG | GCC | CTG | GGC | TGC | CTG | GTC | AAG | GAC | TAC | TTC | CCC | GAA | CCG | GTG | ACG |
| | 504 | | 513 | | | 522 | | | 531 | | | 540 | | | 549 | | | 558 | |
| S | W | N | S | G | A | L | T | S | G | V | H | T | F | P | A | V | L | Q | S |
| TCG | TGG | AAC | TCA | GGC | GCC | CTG | ACC | AGC | GGC | GTG | CAC | ACC | TTC | CCG | GCT | GTC | CTA | CAG | TCC |
| | 567 | | 576 | | | 585 | | | 594 | | | 603 | | | 612 | | | 621 | |
| G | L | Y | S | L | S | S | V | V | T | V | P | S | S | S | L | G | T | Q | T |
| GGA | CTC | TAC | TCC | CTC | AGC | AGC | GTG | ACC | GTG | CCC | TCC | TCC | AGC | AGC | TTG | GGC | ACC | CAG | ACC |
| | 630 | | 639 | | | 648 | | | 657 | | | 666 | | | 675 | | | 684 | |
| I | C | N | V | N | H | K | P | S | N | T | K | V | D | K | K | A | E | P | K |
| ATC | TGC | AAC | GTG | AAT | CAC | AAG | CCC | AGC | AAC | ACC | AAG | GTG | GAC | AAG | AAA | GCA | GAG | CCC | AAA |
| | 693 | | 702 | | | 711 | | | 720 | | | 729 | | | 738 | | | 747 | |
| C | D | K | T | H | T | C | P | P | C | P | A | P | E | L | L | G | G | P | S |
| TGT | GAC | AAA | ACT | CAC | ACA | TGC | CCA | CCG | TGC | CCA | GCA | CCT | GAA | CTC | CTG | GGG | GGA | CCG | TCA |
| | 756 | | 765 | | | 774 | | | 783 | | | 792 | | | 801 | | | 810 | |
| F | L | F | P | P | K | P | K | D | T | L | M | I | S | R | T | P | E | V | T |
| TTC | CTC | TTC | CCC | CCA | AAA | CCC | AAG | GAC | ACC | CTC | ATG | ATC | TCC | CGG | ACC | CCT | GAG | GTC | ACA |
| | 819 | | 828 | | | 837 | | | 846 | | | 855 | | | 864 | | | 873 | |
| V | V | V | D | V | S | H | E | D | P | E | V | K | F | N | W | Y | V | D | G |
| GTG | GTG | GTG | GAC | GTG | AGC | CAC | GAA | GAC | CCT | GAG | GTC | AAG | TTC | AAC | TGG | TAC | GTG | GAC | GGC |
| | 882 | | 891 | | | 900 | | | 909 | | | 918 | | | 927 | | | 936 | |

FIGURE 4b (Continued)

| | | | | | | | | | | | | | | | | | | | | |
|------|-----|-----|-----|------|-----|-----|------|-----|-----|------|-----|-----|------|-----|------|-----|-----|-----|------|-----|
| E | V | H | N | A | K | T | K | P | R | E | E | Q | Y | N | S | T | Y | R | V | V |
| GAG | GTG | CAT | AAT | GCC | AAG | ACA | AAG | CCG | CGG | GAG | GAG | CAG | TAC | AAC | AGC | ACG | TAC | CGT | GTG | GTC |
| 945 | | | | 954 | | | 963 | | | 972 | | | 981 | | 990 | | | | 999 | |
| S | V | L | T | V | L | H | Q | D | W | L | N | G | K | E | Y | K | C | K | V | S |
| AGC | GTG | CTC | ACC | GTC | CTG | CAC | CAG | GAC | TGG | CTG | AAT | GGC | AAG | GAG | TAC | AAG | TGC | AAG | GTC | TCC |
| 1008 | | | | 1017 | | | 1026 | | | 1035 | | | 1044 | | 1053 | | | | 1062 | |
| N | K | A | L | P | A | P | I | E | K | T | I | S | K | A | K | G | Q | P | R | E |
| AAC | AAA | GCC | CTC | CCA | GCC | CCC | ATC | GAG | AAA | ACC | ATC | TCC | AAA | GCC | AAA | GGG | CAG | CCC | CGA | GAA |
| 1071 | | | | 1080 | | | 1089 | | | 1098 | | | 1107 | | 1116 | | | | 1125 | |
| P | Q | V | Y | T | L | P | P | S | R | D | E | L | T | K | N | Q | V | S | L | T |
| CCA | CAG | GTG | TAC | ACC | CTG | CCC | CCA | TCC | CGG | GAT | GAG | CTG | ACC | AAG | AAC | CAG | GTC | AGC | CTG | ACC |
| 1134 | | | | 1143 | | | 1152 | | | 1161 | | | 1170 | | 1179 | | | | 1188 | |
| C | L | V | K | G | F | Y | P | S | D | I | A | V | E | W | E | S | N | G | Q | P |
| TGC | CTG | GTC | AAA | GGC | TTC | TAT | CCC | AGC | GAC | ATC | GCC | GTG | GAG | TGG | GAG | AGC | AAT | GGG | CAG | CCG |
| 1197 | | | | 1206 | | | 1215 | | | 1224 | | | 1233 | | 1242 | | | | 1251 | |
| E | N | N | Y | K | T | T | P | P | V | L | D | S | D | G | S | F | F | L | Y | S |
| GAG | AAC | AAC | TAC | AAG | ACC | ACG | CCT | CCC | GTG | CTG | GAC | TCC | GAC | GGC | TCC | TTC | TTC | CTC | TAC | AGC |
| 1260 | | | | 1269 | | | 1278 | | | 1287 | | | 1296 | | 1305 | | | | 1314 | |
| K | L | T | V | D | K | S | R | W | Q | Q | G | N | V | F | S | C | S | V | M | H |
| AAG | CTC | ACC | GTG | GAC | AAG | AGC | AGG | TGG | CAG | CAG | GGG | AAC | GTC | TTC | TCA | TGC | TCC | GTG | ATG | CAT |
| 1323 | | | | 1332 | | | 1341 | | | 1350 | | | 1359 | | 1368 | | | | 1377 | |
| E | A | L | H | N | H | Y | T | Q | K | S | L | S | L | S | P | G | K | . | | |
| GAG | GCT | CTG | CAC | AAC | CAC | TAC | ACG | CAG | AAG | AGC | CTC | TCC | CTG | TCT | CCG | GGT | AAA | TGA | | |
| 1386 | | | | 1395 | | | 1404 | | | 1413 | | | 1422 | | 1431 | | | | | |

9/16

FIGURE 5a

Length of 16S10 Lambda/Primatized : 711 bp;
Listed from: 1 to: 711;
Translated from: 1 to: 709 (Entire region);
Genetic Code used: Universal; Fri, May 26, 1995 11:08 AM

| | | | | | | | | | | | | | | | | | | | | |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| frame | 1 | M | R | V | P | A | Q | L | L | G | L | L | L | W | L | P | G | A | R | |
| | ATG | AGG | GTC | CCC | GCT | CAG | CTC | CTG | GGG | CTC | CTG | CTG | CTC | TGG | CTC | CCA | GGT | GCA | CGA | |
| | | | 9 | | | 18 | | | 27 | | | 36 | | | 45 | | | 54 | | |
| <hr/> | | | | | | | | | | | | | | | | | | | | |
| C | E | S | V | L | T | Q | P | P | S | V | S | G | A | P | G | Q | K | V | T | I |
| IGT | GAG | TCT | GTC | CTG | ACA | CAG | CCG | CCC | TCA | GTG | TCT | GGG | GCC | CCA | GGG | CAG | AAG | GTC | ACC | ATC |
| | 63 | | | 72 | | | 81 | | | 90 | | | 99 | | | 108 | | | 117 | |
| <hr/> | | | | | | | | | | | | | | | | | | | | |
| S | C | T | G | S | T | S | N | I | G | G | Y | D | L | H | W | Y | Q | Q | L | P |
| TCG | TGC | ACT | GGG | AGC | ACC | TCC | AAC | ATT | GGA | GGT | TAT | GAT | CTA | CAT | TGG | TAC | CAG | CAG | CTC | CCA |
| | 126 | | | 135 | | | 144 | | | 153 | | | 162 | | | 171 | | | 180 | |
| <hr/> | | | | | | | | | | | | | | | | | | | | |
| G | T | A | P | K | L | L | I | Y | D | I | N | K | R | P | S | G | I | S | D | R |
| GGA | ACG | GCC | CCC | AAA | CTC | CTC | ATC | TAT | GAC | ATT | AAC | AAG | CGA | CCC | TCA | GGA | ATT | TCT | GAC | CGA |
| | 189 | | | 198 | | | 207 | | | 216 | | | 225 | | | 234 | | | 243 | |
| <hr/> | | | | | | | | | | | | | | | | | | | | |
| F | S | G | S | K | S | G | T | A | A | S | L | A | I | T | G | L | Q | T | E | D |
| TTC | TCT | GGC | TCC | AAG | TCT | GGT | ACC | GCG | GCC | TCC | CTG | GCC | ATC | ACT | GGG | CTC | CAG | ACT | GAG | GAT |
| | 252 | | | 261 | | | 270 | | | 279 | | | 288 | | | 297 | | | 306 | |
| <hr/> | | | | | | | | | | | | | | | | | | | | |
| E | A | D | Y | Y | C | Q | S | Y | D | S | S | L | N | A | Q | V | F | G | G | G |
| GAG | GCT | GAT | TAT | TAC | TGC | CAG | TCC | TAT | GAC | AGC | AGC | CTG | AAT | GCT | CAG | GTA | TTC | GGA | GCA | GGG |
| | 315 | | | 324 | | | 333 | | | 342 | | | 351 | | | 360 | | | 369 | |
| <hr/> | | | | | | | | | | | | | | | | | | | | |
| T | R | L | T | V | L | G | Q | P | K | A | A | P | S | V | T | L | F | P | P | S |
| ACC | CGG | CTG | ACC | GTC | CTA | GGT | CAG | CCC | AAG | GCT | GCC | CCC | TCG | GTC | ACT | CTG | TTC | CCG | CCC | TCC |
| | 378 | | | 387 | | | 396 | | | 405 | | | 414 | | | 423 | | | 432 | |
| <hr/> | | | | | | | | | | | | | | | | | | | | |
| S | E | E | L | Q | A | N | K | A | T | L | V | C | L | I | S | D | F | Y | P | G |
| TCT | GAG | GAG | CTT | CAA | GCC | AAC | AAG | GCC | ACA | CTG | GTG | TGT | CTC | ATA | AGT | GAC | TTC | TAC | CCG | GGA |
| | 441 | | | 450 | | | 459 | | | 468 | | | 477 | | | 486 | | | 495 | |
| <hr/> | | | | | | | | | | | | | | | | | | | | |
| A | V | T | V | A | W | K | A | D | S | S | P | V | K | A | G | V | E | T | T | T |
| GCC | GTG | ACA | GTG | GCC | TGG | AAG | GCA | GAT | AGC | AGC | CCC | GTC | AAG | GCG | GGA | GTG | GAG | ACC | ACC | ACA |
| | 504 | | | 513 | | | 522 | | | 531 | | | 540 | | | 549 | | | 558 | |
| <hr/> | | | | | | | | | | | | | | | | | | | | |
| P | S | K | Q | S | N | N | K | Y | A | A | S | S | Y | L | S | L | T | P | E | Q |
| CCC | TCC | AAA | CAA | AGC | AAC | AAC | AAG | TAC | GCG | GCC | AGC | AGC | TAC | CTG | AGC | CTG | ACG | CCT | GAG | CAG |
| | 567 | | | 576 | | | 585 | | | 594 | | | 603 | | | 612 | | | 621 | |
| <hr/> | | | | | | | | | | | | | | | | | | | | |
| W | K | S | H | R | S | Y | S | C | Q | V | T | H | E | G | S | T | V | E | K | T |
| TGG | AAG | TCC | CAC | AGA | AGC | TAC | AGC | TGC | CAG | GTC | ACG | CAT | GAA | GGG | AGC | ACC | GTG | GAG | AAG | ACA |
| | 630 | | | 639 | | | 648 | | | 657 | | | 666 | | | 675 | | | 684 | |
| <hr/> | | | | | | | | | | | | | | | | | | | | |
| V | A | P | T | E | C | S | | | | | | | | | | | | | | |
| GTG | GCC | CCT | ACA | GAA | TGT | TCA | TGA | | | | | | | | | | | | | |
| | 693 | | | 702 | | | 711 | | | | | | | | | | | | | |

10/16

FIGURE 5b

Length of 16C10 heavy/primatized: 1431 bp;

Listed from: 1 to: 1431;

Translated from: 1 to: 1429 (Entire region);

Genetic Code used: Universal; Fri, May 26, 1995 11:08 AM

| | | | | | | | | | | | | | | | | | | | | | |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| name 1 | M | K | H | L | W | F | F | L | L | L | V | A | A | P | R | W | V | L | S | | |
| | ATG | AAA | CAC | CTG | TGG | TTC | TTC | CTC | CTC | CTG | GTG | GCA | GCT | CCC | AGA | TGG | GTG | CTG | TCC | | |
| | | | 9 | | | 18 | | | 27 | | | 36 | | | 45 | | | 54 | | | |
| 2 | V | Q | L | Q | E | S | G | P | G | L | V | K | P | S | E | T | L | S | L | T | |
| | CAG | GTG | CAG | CTG | CAG | GAG | TGG | GGC | CCA | GGA | CTG | GTG | AAG | CCT | TCG | GAG | ACC | CTG | TCC | CTC | ACC |
| | 63 | | | 72 | | | 81 | | | 90 | | | 99 | | | 108 | | | 117 | | |
| 3 | A | V | S | G | G | S | I | S | G | G | Y | G | W | G | W | I | R | Q | P | P | |
| | TGC | GCT | GTC | TCT | GGT | GGC | TCC | ATC | AGC | GGT | GGT | TAT | GGC | TGG | GGC | TGG | ATC | CGC | CAG | CCC | CCA |
| | 126 | | | 135 | | | 144 | | | 153 | | | 162 | | | 171 | | | 180 | | |
| 4 | K | G | L | E | W | I | G | S | F | Y | S | S | S | G | N | T | Y | Y | N | P | |
| | GGG | AAG | GGG | CTG | GAG | TGG | ATT | GGG | AGT | TTC | TAT | AGT | AGT | AGT | GGG | AAC | ACC | TAC | TAC | AAC | CCC |
| | 189 | | | 198 | | | 207 | | | 216 | | | 225 | | | 234 | | | 243 | | |
| 5 | L | K | S | Q | V | T | I | S | T | D | T | S | K | N | Q | F | S | L | K | L | |
| | TCC | CTC | AAG | AGT | CAA | GTC | ACC | ATT | TCA | ACA | GAC | ACG | TCC | AAG | AAC | CAG | TTC | TCC | CTG | AAG | CTG |
| | 252 | | | 261 | | | 270 | | | 279 | | | 288 | | | 297 | | | 306 | | |
| 6 | S | M | T | A | A | D | T | A | V | Y | Y | C | V | R | D | R | L | F | S | V | |
| | AAC | TCT | ATG | ACC | GCC | GCG | GAC | ACG | GCC | GTG | TAT | TAC | TGT | GTG | AGA | GAT | CGT | CTT | TTT | TCA | GTT |
| | 315 | | | 324 | | | 333 | | | 342 | | | 351 | | | 360 | | | 369 | | |
| 7 | G | M | V | Y | N | N | W | E | D | V | W | G | P | G | V | L | V | T | V | S | |
| | GTT | GGA | ATG | GTT | TAC | AAC | AAC | TGG | TTC | GAT | GTG | TGG | GGC | CCG | GGA | GTG | CTG | GTG | ACC | GTG | TCC |
| | 378 | | | 387 | | | 396 | | | 405 | | | 414 | | | 423 | | | 432 | | |
| 8 | A | S | T | K | G | P | S | V | F | P | L | A | P | S | S | K | S | T | S | G | |
| | TCA | GCT | AGC | ACC | AAG | GGC | CCA | TGG | GTC | TTC | CCC | CTG | GCA | CCC | TCC | TCC | AAG | AGC | ACC | TCT | GGG |
| | 441 | | | 450 | | | 459 | | | 468 | | | 477 | | | 486 | | | 495 | | |
| 9 | T | A | A | L | G | C | L | V | K | D | Y | F | P | E | P | V | T | V | S | W | |
| | GGC | ACA | GCG | GCC | CTG | GGC | TGC | CTG | GTG | AAG | GAC | TAC | TTC | CCC | GAA | CCG | GTG | ACG | GTG | TCG | TGG |
| | 504 | | | 513 | | | 522 | | | 531 | | | 540 | | | 549 | | | 558 | | |
| 10 | S | G | A | L | T | S | G | V | H | T | F | P | A | V | L | Q | S | S | G | L | |
| | AAC | TCA | GGC | GCC | CTG | ACC | AGC | GGC | GTG | CAC | ACC | TTC | CCG | GCT | GTG | CTA | CAG | TCC | TCA | GGA | CTC |
| | 567 | | | 576 | | | 585 | | | 594 | | | 603 | | | 612 | | | 621 | | |
| 11 | S | L | S | S | V | V | T | V | P | S | S | S | L | G | T | Q | T | Y | I | C | |
| | TAC | TCC | CTC | AGC | AGC | GTG | GTG | ACC | GTG | CCC | TCC | AGC | AGC | TTG | GGC | ACC | CAG | ACC | TAC | ATC | TGC |
| | 630 | | | 639 | | | 648 | | | 657 | | | 666 | | | 675 | | | 684 | | |
| 12 | V | N | H | K | P | S | N | T | K | V | D | K | K | A | E | P | K | S | C | D | |
| | AAC | GTG | AAT | CAC | AAG | CCC | AGC | AAC | ACC | AAG | GTG | GAC | AAG | AAA | GCA | GAG | CCC | AAA | TCT | TGT | GAC |
| | 693 | | | 702 | | | 711 | | | 720 | | | 729 | | | 738 | | | 747 | | |
| 13 | T | H | T | C | P | P | C | P | A | P | E | L | L | G | G | P | S | V | F | L | |
| | AAA | ACT | CAC | ACA | TGC | CCA | CCG | TGC | CCA | GCA | CCT | GAA | CTC | CTG | GGG | GGA | CCG | TCA | GTG | TTC | CTC |
| | 756 | | | 765 | | | 774 | | | 783 | | | 792 | | | 801 | | | 810 | | |
| 14 | P | P | K | P | K | D | T | L | M | I | S | R | T | P | E | V | T | C | V | V | |
| | TTC | CCC | CCA | AAA | CCC | AAG | GAC | ACC | CTC | ATG | ATC | TCC | CGG | ACC | CCT | GAG | GTG | ACA | TGC | GTG | GTG |
| | 819 | | | 828 | | | 837 | | | 846 | | | 855 | | | 864 | | | 873 | | |
| 15 | D | V | S | H | E | D | P | E | V | K | F | N | W | Y | V | D | G | V | E | V | |
| | GTG | GAC | GTG | AGC | CAC | GAA | GAC | CCT | GAG | GTG | AAG | TTC | AAC | TGG | TAC | GTG | GAC | GGC | GTG | GAG | GTG |

11/16

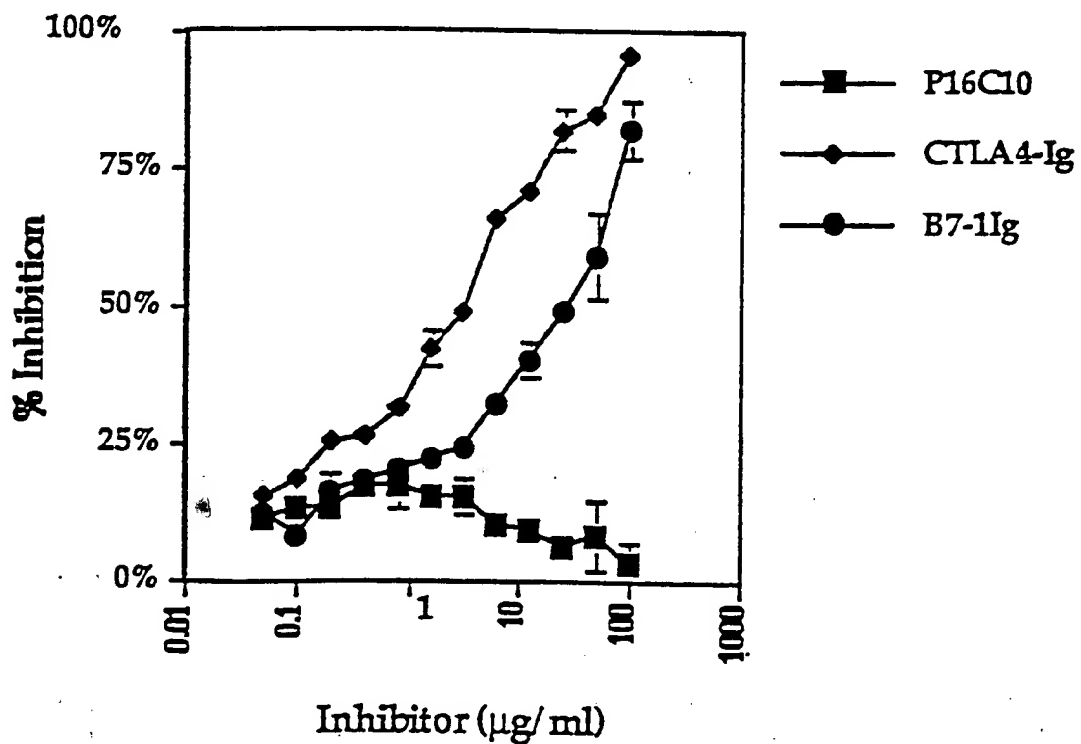
FIGURE 5b (Continued)

| | | | | | | |
|---|------|------|------|------|------|------|
| 882 | 891 | 900 | 909 | 918 | 927 | 936 |
| I N A K T K P R E E Q Y N S T Y R V V S V | | | | | | |
| CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC | | | | | | |
| 945 | 954 | 963 | 972 | 981 | 990 | 999 |
| L T V L H Q D W L N G K E Y K C K V S N K | | | | | | |
| CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA | | | | | | |
| 1008 | 1017 | 1026 | 1035 | 1044 | 1053 | 1062 |
| A L P A P I E K T I S K A K G Q P R E P Q | | | | | | |
| GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG | | | | | | |
| 1071 | 1080 | 1089 | 1098 | 1107 | 1116 | 1125 |
| V Y T L P P S R D E L T K N Q V S L T C L | | | | | | |
| GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG | | | | | | |
| 1134 | 1143 | 1152 | 1161 | 1170 | 1179 | 1188 |
| V K G F Y P S D I A V E W E S N G Q P E N | | | | | | |
| GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC | | | | | | |
| 1197 | 1206 | 1215 | 1224 | 1233 | 1242 | 1251 |
| N Y K T T P P V L D S D G S F F L Y S K L | | | | | | |
| AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC | | | | | | |
| 1260 | 1269 | 1278 | 1287 | 1296 | 1305 | 1314 |
| T V D K S R W Q Q G N V F S C S V M H E A | | | | | | |
| ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT | | | | | | |
| 1323 | 1332 | 1341 | 1350 | 1359 | 1368 | 1377 |
| L H N H Y T Q K S L S L S P G K | | | | | | |
| CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA | | | | | | |
| 1386 | 1395 | 1404 | 1413 | 1422 | 1431 | |

12/16

Figure 6

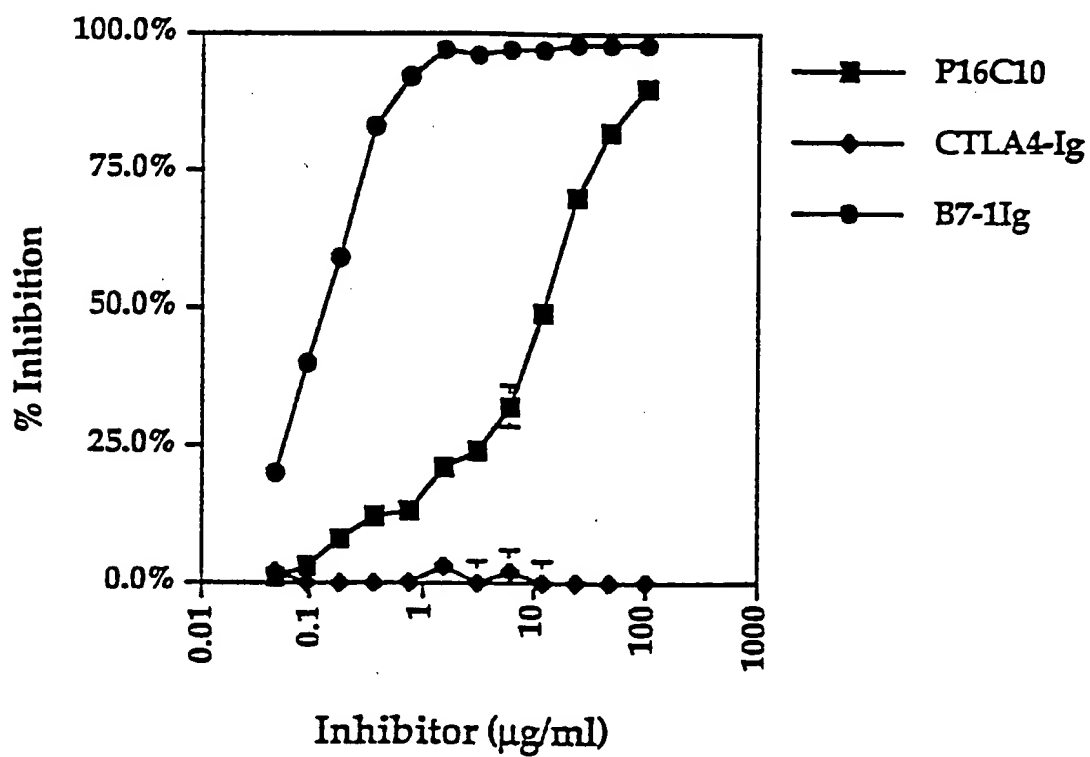
Competitive Binding of CTLA-4Ig-Biotin to B7-1 Transfected CHO Cells in Presence of P16C10 MAb, CTLA-4Ig and B7-1Ig Fusion Proteins



13/16

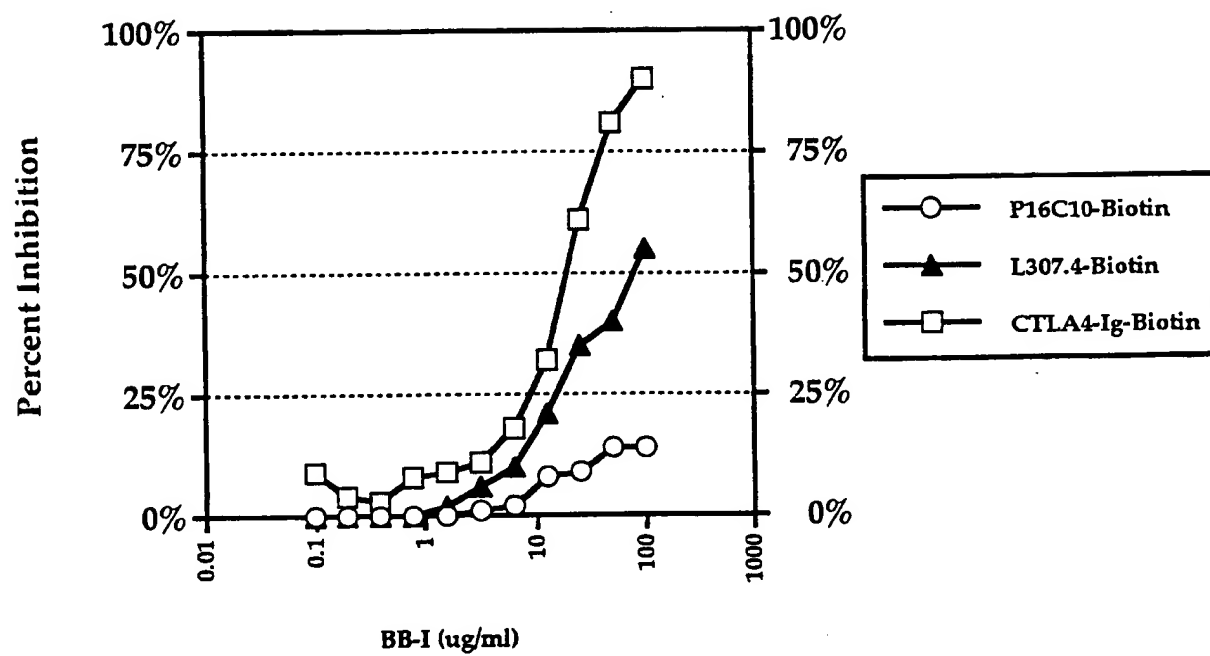
Figure 7

Competitive Binding of P16C10-Biotin to B7-1 Transfected CHO Cells
in Presence of P16C10 MAb, CTLA-4Ig and B7-1Ig Fusion Proteins



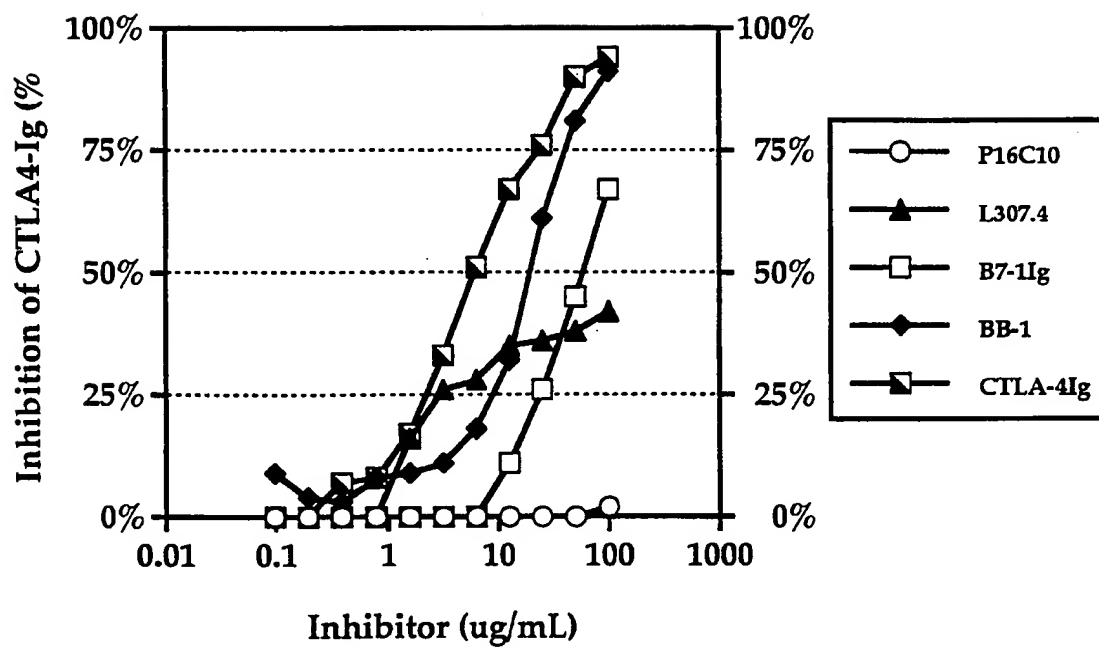
14/16

Figure 8



15/16

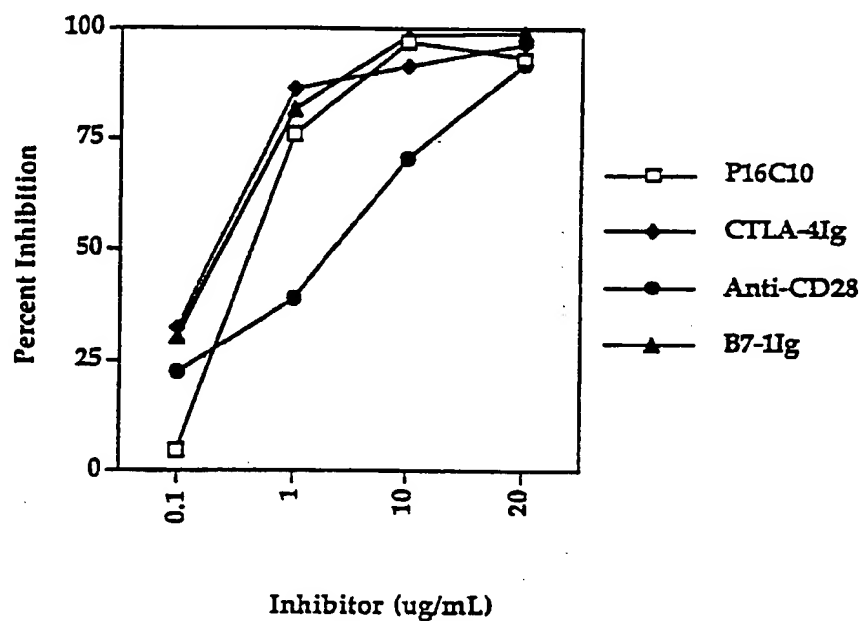
Figure 9



16/16

Figure 10

Competitive Inhibition of Radiolabeled B7-1Ig Binding to CD28 Receptors on Isolated Non-Activated Peripheral Blood T Cells using Unlabeled Binding Proteins Recognizing Either B7-1 or CD28



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/19906

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/395; C07K 16/18, 16/28

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1301., 142.1, 143.1, 144.1, 153.1, 154.1, 173.1; 530/387.1, 387.3, 388.1, 388.15, 388.22, 388.7, 388.73, 388.75

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, BIOSIS, CA, EMBASE, MEDLINE, WPI
search terms: b7.1, clla4, cd28, graft?, transplant?, gvhd**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------------|
| Y | LINSLEY et al. Binding of the B Cell Activation Antigen B7 to CD28 Costimulates T Cell Proliferation and Interleukin 2 mRNA Accumulation. J. Exp. Med. March 1991, Volume 173, pages 721-730, see entire document. | 1-3, 6, 7, 9-14, 16, 18, 26 |
| Y | LINSLEY et al. T-Cell Antigen CD28 Mediates Adhesion With B Cells by Interacting with Activation Antigen B7/BB-1. Proc. Natl. Acad. Sci. USA. July 1990, Volume 87, pages 5031-5035, see entire document. | 1-3, 6, 7, 9-14, 16, 18, 26 |
| Y | US 5,434,131 A (P.S. LINSLEY et al.) 18 July 1995, see entire document. | 1-3, 6, 7, 9-14, 16, 18, 26 |



Further documents are listed in the continuation of Box C.



See patent family annex.

| | |
|---|--|
| * Special categories of cited documents: | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| *A* document defining the general state of the art which is not considered to be of particular relevance | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| *B* earlier document published on or after the international filing date | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *A* document member of the same patent family |
| *O* document referring to an oral disclosure, use, exhibition or other means | |
| *P* document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

07 JANUARY 1998

Date of mailing of the international search report

03 FEB 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

PHILLIP GAMBEL

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/19906

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------------------|
| Y | US 5,521,288 A (P.S. LINSLEY et al.) 28 May 1996, see entire document. | 1-3, 6, 7, 9-14, 16, 18, 26 |
| | | |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/19906

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 6, 7, 9-14, 16, 18, 26

Remark on Protest: ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/19906

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/1301., 142.1, 143.1, 144.1, 153.1, 154.1, 173.1; 530/387.1, 387.3, 388.1, 388.15, 388.22, 388.7, 388.73, 388.75

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This International Search Authority has found 28 inventions claimed in the International Application covered by the claims indicated below and it considers that the International Application does not comply with the requirements of Unity of Invention (Rules 13.1, 13.2 and 3.3 for the reasons indicated below:

This application contains claims directed to the following distinct species of the claimed invention; wherein the antibody specificity is:

- A) B7.1, claims 1-3, 6, 7, 9-14, 16, 18, 20, 22, 24, 26, 27 or
- B) B7.2., claims 1, 4-6, 8-10, 12, 13, 15, 17, 19, 21, 23, 25, 26, 28

These species do not share a common technical feature as these molecules differ in their structures and modes of action; therefore they are distinct.

In addition, this application contains claims directed to the following distinct species of the claimed invention and the specificity of B7.1 or B7.2; wherein the disease is:

- A) thrombocytopenia, claims 20, 21
- B) lupus, claims 20, 21
- C) diabetes, claims 20, 21
- D) arthritis, claims 20, 21
- E) psoriasis, claims 20, 21
- F) anemia, claims 20, 21
- G) IBD, claims 20, 21
- H) allergy, claims 20, 21
- I) multiple sclerosis,
- J) GVHD, claims 22, 23
- K) B cell lymphoma, claims 24, 25
- L) infectious diseases, claims 24, 25
- M) inflammatory diseases, claims 24, 25 or
- N) autoimmunity, claims 18,19.

These species do not share a common technical feature as these pathological conditions differ in etiologies and therapeutic endpoints; therefore they are distinct.

Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.